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(54) Title: TREPONEMA HYODYSENTERIAE ANTIGENS HAVING A MOLECULAR WEIGHT OF 39kDa AND DNA ENCODING THEREFOR

(57) Abstract

A family of *T. hyodysenteriae* 39 kDa antigens are produced by recombinant techniques. Seven different genes and antigens have been identified. Such antigens may be used in a vaccine.

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TREPONEMA HYODYSENTERIAE ANTIGENS HAVING A MOLECULAR WEIGHT OF 39kDa AND DNA ENCODING THEREFOR

This invention relates to Treponema hyodysenteriae and more particularly to Treponema hyodysenteriae (T. hyo.) antigens, genes encoding for such antigens, cells genetically engineered with DNA encoding for such antigens and uses for such antigens. Still more particularly, this invention relates to Treponema hyodysenteriae antigens having a molecular weight of 39kDa and to the production thereof by recombinant techniques.

Swine dysentery is a severe, infectious disease found in all major pig-rearing countries. The symptoms of swine dysentery are severe mucohemorrhagic diarrhea, dehydration and weight loss.

The present invention is directed to certain antigens which are useful in determining and/or treating Treponema hyodysenteriae and to recombinant or genetic engineering techniques for producing such antigens.

In accordance with one aspect of the present invention, there is provided a protein which is

capable of eliciting at least one antibody which recognizes an epitope of at least one T.hyo antigen having a molecular weight of about 39kDa.

Applicant has found that T.hyo includes DNA which encodes for a plurality of proteins each having a molecular weight of about 39kDa. Still more particularly, Applicant has found that there are at least eight different genes, each of which encodes for a T.hyo protein having a molecular weight of about 39 kDa. The protein products encoded by such genes have been found to have conserved regions which are interspersed with variable regions. It has been found that the variable regions are generally located in the more hydrophilic portions of the protein whereas the conserved regions are located in the more hydrophobic portions of the protein.

A comparison of the predicted amino acid sequences from the mature peptides encoded by the 39kDa family genes is found in Appendix 1. The peptide sequence corresponding to the signal peptides of these proteins (Appendices 2A and 2B) has been removed for the purposes of this comparison. Examination of this comparison reveals each gene encodes a protein product of simlar molecular weight and that there are regions of conserved protein sequence punctuated by regions of variable sequence. The conserved regions are generally in the more hydrophobic portions of the proteins while the variable regions tend to be in the more hydrophilic portions. (Kyte & Doolittle, Journal of Molecular Biology, 157, 105 (1982))

Thus, in accordance with one aspect of the present invention, there is provided eight different antigens (or fragments or analogs thereof), which are T.hyo antigens which have a molecular weight of about

39 kDa. Such seven genes are hereinafter sometimes referred to as genes 1-8 or copies 1-8.

In accordance with another aspect of the present invention, there is provided at least eight different genes, each of which encodes for a different T.hyo antigen having a molecular weight of about 39 kDa.

In accordance with yet another aspect of the present invention, there is provided an expression or cloning vehicle which includes a DNA sequence which encodes for a T.hyo antigen (or fragment or analog thereof), which has a molecular weight of about 39 kDa.

In accordance with yet a further aspect of the present invention, there is provided a host cell or organism which is genetically engineered with DNA which encodes for a T.hyo antigen (or fragment or derivative thereof), which has a molecular weight of about 39 kDa.

The molecular weight for characterizing the 39 kDa T. hyo. antigen or protein is obtained by discontinuous polyacrylamide gel electrophoresis using the SDS buffer system described by Laemmli, Nature, 227:680-85 (London, 1970) with an acrylamide concentration of 10-17% and a bis-acrylamide to acrylamide ratio of 1:29.

Thus, Applicant has found that there are at least eight different T.hyo antigens, each of which has a molecular weight of about 39 kDa, which are encoded by eight different genes.

The DNA sequence may encode for a protein which is the entire 39 kDa antigen, or a fragment or derivative of the antigen, or a fusion product of the antigen or fragment and another protein, provided that the protein which is produced from such DNA sequence elicits antibodies after immunization which

recognize an epitope of a 39 kDa T. hyo. antigen. Thus, for example, the DNA sequence may encode for a protein which is or contains within it a fragment of a 39 kDa antigen provided that such fragment generates antibodies which recognize an epitope of a 39 kDa antigen.

Similarly, the DNA sequence may encode for a protein which is a derivative of the antigen e.g., a mutation of one or more amino acids in the peptide chain, as long as such derivative elicits antibodies which recognize an epitope(s) of a 39 kDa T.hyo. antigen as hereinabove described.

The DNA sequence may encode a protein which is a fusion product of (i) a protein which produces antibodies which recognize an epitope(s) of a noted 39 kDa T.hyo. antigen and (ii) another protein (for example chymosin).

The 39 kDa antigens may vary somewhat between specific strains of T. hyo. Thus, for example, the 39 kDa proteins of serotype B204 have minor differences from those of serotype B234; however, such antigens, as well as the genes encoding such antigens are essentially identical to each other.

As a result, the term "DNA sequence which encodes for a protein which produces antibodies which recognize an epitope(s) of a noted 39 kDa T. hyo. antigen" encompasses DNA sequences which encode for and/or express in appropriate transformed cells, proteins which may be the appropriate antigen, antigen fragment, antigen derivative or a fusion product of such antigen, antigen fragment or antigen derivative with another protein.

It is also to be understood that the DNA sequence present in the vector when introduced into a cell may express only a portion of the protein which

is encoded by such DNA sequence, and such DNA sequence is within the noted terminology, provided that the protein portion expressed elicits antibodies which recognize an epitope(s) of one or more of the noted 39 kDa T. hyo. antigens. For example, the DNA sequence may encode for the entire antigen; however, the expressed protein is a fragment of the antigen.

The term "gene (1, 2, 3, 4, 5, 6, 7 or 8) encoding a T. hyo. 39 kDa protein" means the entire or full length gene sequence or an analog, fragment or derivative thereof which encodes a protein which is capable of eliciting at least one antibody which recognizes at least one epitope of the full length T. hyo. 39 kDa antigen encoded by such full length gene.

The term "protein encoded by gene 1, 2, 3, 4, 5, 6, 7 or 8" means a T. hyo. 39 kDa protein encoded by the entire or full length gene or an analogue, fragment or derivative of such protein which is capable of eliciting at least one antibody which recognizes at least one epitope of the full length T. hyo. 39 kDa antigen encoded by such full length gene.

The term "39 kDa T. hyo. antigen or protein" means a T. hyo. antigen or protein having a molecular weight of about 39 kDa.

The appropriate DNA sequence may be included in any of a wide variety of vectors or plasmids. Such vectors include chromosomal, nonchromosonal and synthetic DNA sequences; e.g., derivatives of SV40; bacterial plasmids; phage DNA's; yeast plasmids; vectors derived from combinations of plasmids and phage DNAs, viral DNA such as vaccinia, adenovirus, fowl pox virus, and pseudorabies.

The appropriate DNA sequence may be inserted into the vector by a variety of procedures. In general, the DNA sequence is inserted into an

appropriate restriction endonuclease site(s) by procedures known in the art. Such procedures and others are deemed to be within the scope of those skilled in the art.

The DNA sequence in the expression vector is operatively linked to an appropriate expression control sequence(s) (promoter) to direct mRNA synthesis. As representative examples of such promoters, there may be mentioned: LTR or SV40 promoter, the E. coli. lac or trp, the phage lambda PL promoter and other promoters known to control expression of genes in prokaryotic or eukaryotic cells or their viruses. The expression vector also contains a ribosome binding site for translation initiation and a transcription terminator. The vector may also include appropriate sequences for amplifying expression.

In addition, the expression vectors preferably contain a gene to provide a phenotypic trait for selection of transformed host cells such as dihydrofolate reductase or neomycin resistance for eukaryotic cell culture, or such as tetracycline or ampicillin resistance in E. coli.

The vector containing the appropriate DNA sequence as hereinabove described, as well as an appropriate promoter or control sequence, may be employed to transform an appropriate host to permit the host to express the protein. As representative examples of appropriate hosts, there may be mentioned: bacterial cells, such as E. coli, Salmonella typhimurium; fungal cells, such as yeast; animal cells such as CHO or Bowes melanoma; plant cells, etc. The selection of an appropriate host is deemed to be within the scope of those skilled in the art from the teachings herein.

As hereinabove indicated, the expression vehicle including the appropriate DNA sequence inserted at the selected site may include a DNA or gene sequence which is not part of the gene coding for the protein which is capable of producing antibodies which recognize an epitope(s) of the noted T. hyo. antigen(s). For example, the desired DNA sequence may be fused in the same reading frame to a DNA sequence which aids in expression or improves purification or permits expression of the appropriate protein.

When seeking to develop a vaccine neutralizing or protective antibodies could be targeted towards discontinuous, conformation-dependent epitopes of the native antigen. One must therefore consider whether the protein obtained from the recombinant expression system might have a three dimensional structure (conformation) which differs substantially from that of the original protein molecule in its natural environment. Thus, dependent on the immunogenic properties of the isolated proteins, one might need to renature it to restore the appropriate molecular conformation. Numerous methods for renaturation of proteins can be found in the scientific literature and include; (1) denaturation (unfolding) of improperly folded proteins using agents such as alkali, chaotropes, organic solvents and ionic detergents followed by a renaturation step achieved by dilution, dialysis, or pH adjustment to remove the denaturant, and (2) reconstitution of proteins into a lipid bilayer or liposome to re-create a membrane like environment for the immunogenic protein.

In accordance with another aspect of the present invention, one or more of the proteins produced from a genetically engineered host (genetically engineered

with DNA encoding for a 39 kDa T. hyo antigen) may be employed in conjunction with a pharmaceutically acceptable carrier or may be directly conjugated to a carrier or immunostimulant to provide protection against swine dysentery, and in particular swine dysentery induced by T. hyo.. The Rotavirus VP6 carrier system developed by VIDO (Veterinary Infectious Disease Organization, Saskatoon, Canada) although not an adjuvant may be a suitable immunostimulant when chemically conjugated to a 39 kDa T. hyo. antigen. As hereinabove indicated, such protein(s) is capable of eliciting antibodies which recognize an epitope(s) of one or more of the hereinabove noted 39 kDa T. hyo. antigens. Such expressed protein will be sometimes hereinafter referred to as a "recombinant T. hyo. antigen," however, as hereinabove indicated, such protein may not correspond to a T. hyo. antigen in that it may also be a fragment, derivative or fusion product. The term "recombinant T. hyo. antigen" also encompasses such fragments, derivatives and fusion products.

One or more of such 39kDa T. hyo. antigens may be employed in the vaccine. In a preferred embodiment, all of the 39kDa T. hyo. antigens are employed in formulating a vaccine (i.e., the seven antigens or fragments or derivatives thereof encoded by the seven different T. hyo. genes).

The recombinant T. hyo. antigen(s) is employed in the vaccine in an amount effective to provide protection against swine dysentery. In general, each dose of the vaccine contains at least 5 micrograms and preferably at least 20 micrograms of such recombinant T. hyo. antigen(s). In most cases, the

vaccine does not include such recombinant T. hyo. antigen in an amount greater than 20 milligrams.

The term "protection" or "protecting" when used with respect to the vaccine for swine dysentery described herein means that the vaccine prevents swine dysentery and/or reduces the severity of swine dysentery.

If multiple doses are given, in general, they would not exceed 3 doses over a six week period.

The vehicle which is employed in conjunction with the recombinant T. hyo. antigen(s) may be any one of a wide variety of vehicles. As representative examples of suitable carriers, there may be mentioned: mineral oil, alum, synthetic polymers, etc. vehicles for vaccines are well known in the art and the selection of a suitable vehicle is deemed to be within the scope of those skilled in the art from the teachings herein. The selection of a suitable vehicle is also dependent upon the manner in which the vaccine is to be administered. The vaccine may be in the form of an injectable dose and may be administered intra-muscularly, intravenously, or by sub-cutaneous administration. It is also possible to administer the vaccine intranasally or orally by mixing the active components with feed or water; providing a tablet form, etc.

Other means for administering the vaccine should be apparent to those skilled in the art from the teachings herein; accordingly, the scope of the invention is not limited to a particular delivery form.

It is also to be understood that the vaccine may include active components or adjuvants in addition to the recombinant T. hyo. antigen or fragments thereof hereinabove described.

In accordance with a further aspect of the present invention, there is provided an assay for detection or determination of antibody to 39 kDa T. hyo. antigen which employs a 39 kDa T. hyo. protein antigen, of the type hereinabove described, as a specific binder in the assay.

More particularly, there is provided an immunoassay for 39 kDa T. hyo. antibody in which a 39 kDa T. hyo. antigen is employed as a binder, in the assay, for specifically binding 39 kDa T. hyo. antibody.

The assay technique which is employed is preferably a sandwich type of assay wherein the 39 kDa T. hyo. antigen is supported on a solid support, as a binder, to bind 39 kDa T. hyo. specific antibody present in a sample, with the bound antibody then being determined by use of an appropriate tracer.

The tracer is comprised of a ligand labeled with a detectable label. The ligand is one which is immunologically bound by the 39 kDa T. hyo. antibody and such ligand may be labeled by techniques known in the art.

Thus, for example, the 39 kDa T. hyo. antibody bound to the 39 kDa T. hyo. antigen on the solid support may be determined by the use of an antibody for 39 kDa T. hyo. antibody which is labeled with an appropriate detectable label.

In such a sandwich assay technique, the labeled antibody to 39 kDa T. hyo. antibody may be a monoclonal antibody or a polyclonal antibody; e.g. the polyclonal antibody may be anti-swine IgG or may be an antibody which is specific for 39 kDa T. hyo. antibody, which antibody may be produced by procedures known in the art; for example innoculating an appropriate animal with 39 kDa T. hyo. antibody.

The detectable label may be any of a wide variety of detectable labels, including enzymes, radioactive labels, chromogens (including both fluorescent and/or absorbing dyes) and the like. The selection of a detectable label is deemed to be within scope of those skilled in the art from teachings herein.

The solid support for the antigen may be any one of a wide variety of solid supports and the selection of a suitable support is deemed to be within the scope of those skilled in the art from the teachings herein. For example, the solid support may be a microtiter plate; a tube, a particle, etc.; however, the scope of the invention is not limited to any representative support. The antigen may be supported on the support by techniques known in the art; e.g., by coating; covalent coupling, etc. The selection of a suitable technique is deemd to be within the scope of those skilled in the art from the teachings herein.

The sandwich assay may be accomplished by various techniques; e.g., "forward"; reverse"; or "simultaneous"; however, the forward technique is preferred.

In a typical procedure, 39 kDa T. hyo. antigen, which is supported on a solid support is initially contacted with a sample containing or suspected of containing 39 kDa T. hyo. antibody to bind specifically any of such antibody present in the sample to such antigen on the support.

After washing of the solid support, the support is contacted with a tracer which binds to 39 kDa T. hyo. antibody. If such antibody were present in the sample, the tracer becomes bound to such antibody bound to such antigen on the solid support, and the

presence of tracer on the solid support is indicative of the presence of 39 kDa T. hyo. antibody in the sample. The presence of tracer may be determined by determining the presence of the detectable label by procedures known in the art.

Although the preferred procedure is a sandwich assay, it is to be understood that the 39 kDa T. hyo. antigen(s) may be used in other assay techniques, e.g., an agglutination assay wherein the antigen is used on a solid particle such as a latex particle.

In accordance with another aspect of the present invention, there is provided an assay or reagent kit for determining 39 kDa T. hyo. antibody which includes 39 kDa T. hyo. antigen, as hereinabove described, and a tracer comprised of a ligand and a detectable label. The ligand of the tracer is bound by 39 kDa T. hyo. antibody. The reagents may be included in a suitable kit or reagent package, and may further include other components, such as buffers etc. The 39 kDa T. hyo. antigen is preferably supported on a solid support.

DNA fragments may be used as a probe by use of techniques known in the art.

Although the present invention has been particularly described with reference to use of the 39 kDa antigen(s) for imparting protection against T. hyo., one or more of such antigens may be used to produce antibodies (monoclonal and/or polyclonal) by procedures known in the art and such antibodies may be used in a vaccine to impart protection against T. hyo.

Description of Appendices and Drawings

Appendix 1 is a comparison of Gene Products of the 39kDa gene Family without peptide signal sequences from serotype B204 .

Appendix 2A is the DNA sequence of genes 1-4 encoding antigenes 1-4 of the 39kDa gene family from serotype B204.

Appendix 2B is the DNA sequence of genes 5-8 encoding antigens 5-8 of the 39 kDa gene family from serotype B204.

Appendix 3 is the nucleotide sequence of T.hyo gene insert of pTrep 106.

Appendix 4 is a partial DNA sequence of plasmid pTrep 301.

Appendix 5 is predicted amino acid sequences from PCR derived T.hyo. (B204) clones.

Appendix 6 is the predicted protein seqeunce encoded by pTrep 702.

Appendix 7 is the predicted protein sequence encoded by pTrep 704.

Appendix 8 is the predicted amino acid sequence for pTrep 505.

Figure 1 is a map of the gene family and sub-clones obtained from screening for 39 kDa gene;

Figure 2 is a plasmid map of pTrep 505;

Figure 3 is a schematic of the construction of pTrep 702;

Figure 4 is a schematic of the construction of pTrep 704; and

Figure 5 is a schematic of the construction of the pTrep PCR expression vehicle.

The present invention will be further described with respect to the following examples; however, the scope of the invention is not to be limited thereby. In the Examples, unless otherwise noted, purifications, digestions and ligations are accomplished as described in "Molecular Cloning, a laboratory manual" by Maniatis et al. Cold Spring

Harbor Laboratory (1982). In the following examples, unless otherwise indicted, transformations are accomplished by the procedure of Cohen et al. PNAS 69 2110 (1973).

EXAMPLE I - Purification and Recovery of Native Antigen

Treponema hyodysenteriae strain B204 was grown in broth culture prepared as follows. Brain/Heart Infusion (Difco) at 37 gms/liter distilled water was autoclaved allowed to cool, then sterile additions were made of a glucose solution (to a final concentration of 5 gm/liter) and fetal calf serum (to final concentration of 5% vol/vol). The media was then prereduced (made anaerobic) by 24 hours of perfusion with a stream of gas composed of 90% nitrogen, 10% carbon dioxide. The complete media was then inoculated with a 1-10% volume of actively growing T. hyo culture, the temperature was maintained at 37°C-39°C, the culture pH was maintained at 6.8, and the culture was continuously perfused with the oxygen free gas (flow rate 50 mls/min/liter of culture).

Cells were removed from the fermentation when they had achieved a density of 5×10^8/ml or greater (measured by microscopic count). Cells were concentrated by centrifugation then washed and recentrifuged twice in a buffer of 10mM potassium acetate pH 4.75, 150mM potassium chloride. The cells were then resuspended in 10mM potassium acetate pH 4.75 until an optical density of 25-30 (at 600nm) was achieved (as measured on solution dilutions) which is typically about 1/20 the original culture volume.

Extraction method:

Tween-20 (a non-ionic detergent) was then added to the cell suspension to achieve a final concentration of 0.2%. After gentle agitation for 10 minutes the cells were centrifuged (10,000 xg for 10 min). This supernatant fraction was discarded and the cells were resuspended in an equivalent volume of acetate buffer and then extracted by the addition of Tween-20 to a final concentration of 2.0%. After centrifugation the 2% Tween supernatant (detergent solubilized antigen pool) was saved and the cell pellet was resuspended and re-extracted with Tween-20 in a sequential manner for up to 5 additional cycles with the Tween-20 concentration increasing over the cycles from about 2% up to about 10%. The detergent solubilized supernatant fractions were pooled. This extraction procedure selectively (but not quantitatively) solubilizes surface proteins of T. hyo without lysing or rupturing the bacteria.

To concentrate the antigen preparation, supernatent fractions were subjected to ultracentrifugation (100,000 x g) for 1.5 hours, and the recovered pellet material (HSP) was resuspended in 25mM Tris buffer pH 6.8 and dispersed by sonication.

Antigen Purification

The resuspended HSP was then mixed with 15 volumes of Tris-HCl pH 6.8, 6M urea which had been filtered through a 0.45uM filter. This was stirred at room temperature for several hours. This was centrifuged at 100,000 xg and the supernatant (US1) set aside. The pellet fraction from this step (UP1) was resuspended and extracted with urea a second time. This material was centrifuged as before and the supernatant (US2) and pellet (UP2) were collected.

The predominant protein constitutent of UP2 is 39 KDa protein sometimes referred to as the 39p antigen. The 39p antigen which was further purified by molecular sieve column chromatography in the presence of SDS or electroelution from acrylamide gels.

It is possible to isolate a soluble form of the 39kDa antigen (39s) in addition to the sedimentable form (39p) that is isolated as the major protein component of the urea insoluble pellet (UP2) described above. In order to produce the 39s antigen, T.hyo cells (B204) were extractd with Tween 20 as described above. After the final Tween 20 extraction the residual cell pellet was resuspended in approximately 2ml of 10mM potassium acetate, pH 4.75 per gram wet weight and sonicated. The sonicated cell pellet was separated from the 39s antigen, by centrifugation at 26,000 xg for 15' at 4C. The supernatant was then centrifuged at 100,000 xg for 2 hours at 20°C to pellet any of the sedimentable membrane associated proteins which were also released by sonication. The supernatant (39*), which contains the 39s antigen as its predominant protein component, was then sterile filtered through a .2uM filter and stored at either 4C or frozen. If stored at 4C some proteolytic degredation of the 39s antigen occurs. Additional 39* can be isolated by repeating the above sonication and centrifugation steps on the 26,000 xg cell pellet. Approximately 4mg of 39* can be obtained per liter of original culture volume; a yield roughly equivalent to the yeild of UP2.

The electrophoretic mobility in SDS polyacrylamide gels of the 39s protein and the 39p protein is identical. The two proteins are also

immunologically cross-reactive. Anitsera raised against UP2, or gel purified 39p, will recognize 39s on Western blots. Conversely, antisera raised against 39* will recognize 39p on Western blots. 39S and 39p also comigrate with the predominant protein on the surface of intact T.hyo cells labelled with I^{125}. (Marchalonis, et al. Biochemistry Journal; 24, 921 (1971)). Antisera from swine that have recovered from experimental infections of swine dysentery also recognize either the 39s or 39p form of the 39kDa antigen.

Example 2 - Protein Sequence of 39s and 39 antigens

The fermentation and protein purification are accomplished as in Example 1.

The insoluble material obtained by centrifugation of the second urea extraction (UP2) contains a single major protein component which is 39p antigen. This insoluble protein was solubilized by boiling in 25 mM Tris-HCl pH6.8 containing 3%SDS, 1 mM EDTA, and 70 mM 2-mercaptoethanol. This solution was subjected to gel filtration chromatography over a 30 cm column of Sepharose 6B (from BioRad, Richmond, CA). The 39 kDa peak was identified by gel electrophoresis of column eluant fractions, the appropriate fractions were pooled and the protein concentrated by precipitation with acetone and collected by centrifugation. The pellet was dissolved in 1.1% SDS and then extracted with chloroform/methanol to remove residual SDS.

The amino acid sequence of the amino-terminus of the 39 kDa protein prepared above was determined using sequential Edman degradation in an automated Applied Biosystems gas phase sequenator. The identity of the first 41 amino acids of the protein thus determined are shown below:

1 10
Met-Tyr-Gly-Asp-Arg-Asp-Ser-Trp-Ile-Asp-Phe-Leu-Thr-His-Gly-
20
Asn-Gln-Phe-Arg-Ala-Arg-Met-Asp-Gln-Leu-Gly-Phe-Val-Leu-Gly-
41
Asn-Asp-Thr-Ile-Lys-Gly-Thr-Phe-?-?-Arg-

Amino terminal peptide sequence of 39s was obtained directly from a preparation of 39* which was concentrated by precipitation with acetone and sequenced. Additional internal peptide sequence of the 39s antigen was obtianed by digestion with endoproteinase LysC (~1/100 w/w) in 50mM Tris-HCl pH8.5, 0.1% SDS (37°C, 16 hrs.). Proteolytic cleavage products were purified using reverse phase HPLC and sequenced on a Vydac C4 column (250 mm x 4.6 mm, 5μM) developed with a linear gradient of 0%-100B% (0% = 0.1% Trifluoracetic acid, 100B% = 67% acetonetrile, 33% isopropanol, 0.1% trifluoroacetic acid). Peptide sequence for the 39p antigen extending that found above was determined in a similar fashion. Some additional protein sequence from 39* was also obtained from purified cleavage products after digestion with endoproteinase V8(~1/100 w/w) in 50mM NH_4HCO_3, pH 7.8, 0.1% SDS.

Internal sequences were also determined for the 39p antigen as follows:

An amino acid sequence was determined for an HPLC purified peptide fragment derived from proteolyitc digestion of the 39 kDa protein (of the UP2 cell fraction)using endoproteinase Lys-C. 300μg of the 39 kDa protein was first precipitated with acetone and then resuspended in a solution of 4 M urea, 25 mM Tris pH 8.5 and digested with 2.5μg LysC

(37°C, 16 hrs.). One peptide was obtained as a peak of material eluting off of a C-4 reverse phase column developed with a gradient of acetonitrile, isopropanol (2:1) in 0.1% trifluroacetic acid.

The purified fragment had the following internal sequence: val gln his ser leu ala trp gly ala tyr ala glu leu tyr val arg pro val gln asp leu glu glu tyr phe glu met asp ile asn. . .

The Amino acid sequence was also determined for the protease resistant component of the 39 kDa component of the UP2 fraction after its digestion with chymotrypsin. A sonicated suspension of UP2 protein at 2mg/ml was incubated at 37 degrees C for 16 hrs. with 20 ug/ml chymotrypsin in a buffer of 25 mM Tris, pH 6.8, containing 0.1% Zwittergent 3-12 detergent. A protease resistant 27 kDa product was isolated by electroelution after preparative gel electrophoresis and precipitated and extracted with chloroform/methanol prior to sequencing. The component had the following sequence:

asp xxx xxx thr lys-
asp tyr met gly ile ser thr asp ile gln leu arg tyr tyr thr xxx ile asp ala phe asn ala ile arg leu tyr phe lys tyr gly gln xxx xxx phe

A summary and comparison of the amino acid sequence data obtained from 39p and 39s is found in Table 1 following the Examples. Although the proteins have not been sequenced in their entirety, none of the data identifies any difference in the amino acid sequence of the 39p and 39s antigens. Therefore, one can conclude that the amino acid sequences of the 39p and 39s antigens are very similar and may be identical.

Example 3 - General Methods

Unless otherwise indicated, the General Methods described below were used in the following examples.

A. Construction of a Genomic Library of T.hyo DNA

48 µg of genomic DNA of T.hyo strain B204 was partially digested with Alu I. EcoRI linkers were kinased with P32 ATP according to manufacturers instructions (Pharmacia LKB Biotechnology, Piscataway, New Jersey) and ligated to the Alu I partially digest T.hyo DNA at a linker concentration of 133 µg/ml using BMB ligase at a concentration of 50 units/ml. Following overnight ligation the ligase was heat inactivated and the reaction was digested with EcoRI.

The DNA was fractionated on an S-200 (Pharmacia) column using 0.3 NaCl, 0.05 m Tris-HCl pH 8.0, 1 mM EDTA, 0.06% sodium azide as a column buffer, in order to remove free linkers and free ATP. The recovered T.hyo DNA was then ligated to dephosphorylated lambda gt11 EcoRI arms obtained from PRomega Biotec (Madison, Wisconsin) and used according to manufacturers specifications. The ligation was then packaged into lambda bacteriophage particles using the in vitro packaging kit, "Gigpack,"obtained from Stratagene (San Diego, CA). The phage was then titered on a stationary phage culture of E. coli strain Y1090r-(Promega Biotech). The number of white plaques indicated that the original phage stock contained 1.4 X 10E7 pfu/ml in a total of 0.5ml.

B. Identification of a Recombinant Phage

In performing mixed oligonucleotide screening for the 39kDa gene, the procedure used was that of W.D. Benton & R.W. Davis Science 196, 180 (1977).

Duplicate filters were hybridized with each oligonucleotide probe. Approximately 10E6 cpm (1-2 ng probe) of probe was used for filter, overnight at 37C. The hybridization solution consisted of:

5X Denharts

0.1 um rATP

250 µg/ml E. coli tRNA

6X NET (1XNET=150mM NaCl, 15 mM Tris-HCl pH 7.5, 1 mM EDTA)

0.5% NP40

1 mM sodium Pyrophosphate

Prior to hybridization the filters were washed for 2 hours at 37C in hybridization solution. Following hybridization the filters were washed twice at RT (20'/wash) and twice (20'/wash) at 37C in 6X NET, 0.1% SDS and once (20'/wash) at 37C in 6X NET. The filters were then dried and exposed to X-ray film. Positive plaques were selected, rescreened and plaque purified. Phage DNA was isolated using the technique of C. Helms, et al. (DNA 4 39, 1985).

C. PCR Protocol

1. 10ng genomic DNA (B204 or B234) was mixed with 10ul 10x reaction buffer (Perkin Elmer Cetus), 16ul 1.25mM dNTP (each), 25ul primer#1 (4uM), 25 ul primer #2 (4uM) and brought to a final volume of 100ul with Q-H_2O.

2. The mixture was denatured by heating at 94°C for 1.5 min. and annealed at 50°C for 2.5 min. 0.5ul of Taq polymerase (15U/µl) (Perkin Elmer Cetus) was added and polymerization was allowed to proceed at 55°C for 10 min.

3. One more round of denaturation, annealing and polymerization was performed with the time and temperature conditions specified in step #2.

4. Twenty three rounds of denaturation, annealing and polymerization were performed as in step #2 except that the polymerization temperature was increased to 65°C.

5. After the final round of amplification the mixture was extracted with phenol:chloroform (1:1) and chloroform and precipitated with ethanol.

6. After extraction and precipitation the sample was digested with appropriate enzymes (BamH1 and Hind3) and ligated with the desired vector (pUC8 or pUC9).

D. Dot Blot Screening Protocol

1. Grow an overnight culture of bacterial colony to be screened.

2. Spin down 50ul of the overnight culture and remove supernatant.

3. Resuspend the cell pellet in 200ul of 25mM Tris-Cl pH 8.0, 10mM EDTA + hen egg white lysozyme (1mg/ml).

4. Incubate for 5 min. at room temperature.

5. Sonicate briefly (3 seconds).

6. Add 20ul 3N NaOH and incubate 1 hour at 70C.

7. Let cool to room temperature, add 220ul 2M NH_4OAc. Mix.

8. Apply to nitrocellulose filter with aid of vacuum.

9. Let filter air dry. Bake at 90C for 2hrs.

10. Probe filter with desired probe.

E. Preparation of Nick Translated Probe

1. Denature 50ng of the DNA fragment by boiling for 5 min. in 9ul TE. Chill on ice.

2. Add 5ul gamma dATP (6000 Ci/mMol, 10mCi/ml), 2ul degenerate hexamer in 10x reaction buffer (BMB), 3ul dNTP (25uM dG-,dT-, & dCTP final concentration), 2ul Klenow (2U/ul, BMB).

3. Incubate at 37C for 30 min. Stop with 30ul 10mM EDTA.

4. Separate labeled fragement from unincorporated label with G-50 spin column (BMB).

F. Screening Procedure with Nick Translated Probes

The screening procedure was as follows:

1. Prewash baked nitrocellulose filters in 20mM Tris-Cl pH8.0, 1mM EDTA, 0.1% SDS for 2 hrs. at 37°C.

2. Prehybridize filters for 2 hrs. at 42°C in 50% deionized formamide, 5X Denhardt's, 5X SSPE, 0.1% SDS, salmon sperm DNA (100ug/ml).

3. Denature nick translated probe by boiling for 5 minutes and chilling on ice.

4. Hybridize overnight at 42°C in prehybridization solution and denatured probe.

5. Wash filters 2 times at room temperature in 2X SSC and 0.1% SDS.

6. Wash 1 time in 0.1X SSC at 42°C.

7. Dry filters and expose to x-ray film at -70°C with enhancing screen.

G. Cement Preparation

1. Resuspend cells from an overnight culture in 1/25th original volume in 25mM Tris-Cl pH 8.0, 10mM EDTA + 1mg/ml lysozyme.

2. Incubate 30-60 min. Sonicate to disrupt DNA and reduce viscosity.

3. Add 1/10th volume 20% Triton X-100. Agitate on lab quake for 2 hours. Sonicate if necessary to reduce viscosity.

4. Centrifuge at 10,000xg for 10 min. to pellet cement.

5. Resuspend cement in 1/25th original volume in 20mM Tris-Cl pH 8.0, 5mM EDTA + 5% Triton X-100. Sonicate. Agitate on lab quake overnight.

6. Centrifuge at 10,000xg for 10 min. to pellet cement. Wash cement with 20mM Tris-Cl pH 8.0, 5mM EDTA. Centrifuge.

7. Resuspend in 1/50th original volume in 20mM Tris-Cl pH8.0, 5mM EDTA.

Example 4 - Identification of the Gene Encoding the Initial Member of the 39 kDa Antigen family

A set of DNA probes were synthesized using the amino terminal amino acid sequence data shown in Example 2. Each of them were comprised of a pool of degenerate sequences which encompass all the possible combinations of nucleotides which could encode the amino acid sequence of the target region as indicated below. Each probe is 17 nucleotides in length.

1

```
 Met-Tyr-  Gly-Asp-Arg-Asp
ATG-TAT-GGT-GAT-AGT-GA
       C    C   C   C
            A       A
            G       G
```

probe name = COD 555

degeneracy = 128 fold
(mix of 128 combinations)

10

```
 Trp-Ile-Asp-Phe-Leu-Thr
TGG-ATT-GAT-TTT-TTT-AC
       C    C   C   C
       A            A
                    G
```

probe name = COD 553

degeneracy = 96 fold

18

```
 His- Gly- Asn- Gln- Phe- Arg
CAT-GGT-AAT-CAA-TTT-AG
  C    C    C    G  CC
       A
       G
```

probe name = COD 558

degeneracy = 128 fold

A lambda GT11 library containing EcoRI linkered fragments derived from á partial AluI digest of genomic T.hyo DNA (strain B204 was screened with probes. One phage, 3-5C1 was identified by hybridization to probes 553 and 555. The DNA was examined after digestion with EcoR1 and found to contain a 1.6 kb insert.

The Eco R1 flanked, 1.6 kb segment of DNA from phage 3-5C1 was isolated by electroelution from an acrylamide gel and then ligated to plasmid pUC 19 which had been linearized by digestion with EcoR1. These DNAs were the ligated together, transformed into E. coli, and a clone containing recombinant plasmid pTrep 106 (Appendix 3) was identified by analysis or restriction digests of plasmid DNA.

Plasmid pTrep 106 was used to direct protein synthesis in an in vitro coupled transcription-translation system containing ^{35}S-Methionine. SDS-gel electrophoresis of the

protein products of this system showed 39 kDa protein species not seen with the parental plasmid lacking the T. hyo DNA insert. This suggests that the cloned DNA contains the complete coding sequence for the T. hyo 39 kDa antigen and that E. coli is capable of recognizing the treponemal promoter and ribosome binding site and directing the synthesis of this foreign protein.

E. coli. strains transformed with plasmid pTrep 106 did not produce significant amounts of the desired 39 kDa T. hyo. antigen. Therefore, plasmid construction allowing high level expression of the recombinant antigen was made as follows. The Eco RI flanked, 1.6 kb fragment of pTrep 106 was ligated to plasmid pUC 18 linearized by digestion with Eco RI. The resulting plasmid, pTrep 112, was then cut with PstI and BamHI, then treated with exonuclease III to remove (in a unidirectional manner) the non-coding DNA sequence upstream of the predicted ATG start codon of the 39 kDa T. hyo. antigen (Henikoff, Gene 28 p. 351-59 (1984)). At various times during this digestion, DNA aliquots were removed, the exo III inactivated by phenol extraction, the remaining DNA rendered blunt ended by digestion with nuclease S1, and this DNA was then religated and used to transform E. coli. Nucleotide sequencing (Sanger, et al., PNAS 74:5463 (1977)) of plasmid DNA from one such new clone, pTrep 112-1, indicated that a contiguous sequence of 372 codons encoding the mature T. hyo. 39 kDa protein and 7 amino acids from the signal sequence were fused downstream of the Hind III site of the parental pUC 18 plasmid. The fusion was in a reading frame to encode a fusion protein whose expression would be regulated by the lac promoter after the orientation of the cloned fragment was

inverted (see Appendix 4) by cloning into pUC 9 from the HindIII to Eco RI site. E. coli. transformed with the resulting plasmid, pTrep 301, produced an insoluble 39 kDa antigen which reacts with sera from swine (both those recovered from swine dysentery as well as animals immunized with the 39 kDa protein purified from T. hyo.) in both an immunoblot and plate ELISA assay.

PURIFICATION OF THE RECOMBINANT FORM OF THE 39 KDA ANTIGEN

E. coli strain CY-15,000 containing plasmid pTrep301 was grown in 250 mls of Luria broth containing ampicillin at 200 µg/ml. The culture was grown for 18 hours at 37° C. The cells were harvested by centrifugation then resuspended in 1/20 their original volume in a buffer of 25 mM Tris, 10 mM EDTA at pH 8.0 and containing lysozyme at 1 mg/ml. After a 30 minute incubation at room temperature the cells had lysed and were then further disrupted by sonication. The non-ionic detergent, Triton X-100 was added to a final concentration of 2%, the cell lysate was mixed for 1 hour at room temperature and then centrifuged at 10,000 xg. The insoluble pellet fraction after these steps was saved. The major protein component of this fraction had a Mr of about 40 kDa as judged by Commassie blue staining of samples after SDS-gel electrophoresis. This same protein component was recognized in Western blot analysis by swine and mouse antisera raised against the authentic 39 kDa T. hyo protein obtained from the UP2 fraction. This recombinant protein was also recognized in immunoblots probed with sera from pigs that had recovered from experimentally induced swine dysentery.

The predicted amino acid sequence of the 39kDa recombinant antigen obtained in this Example closely resembles but is not identical to the amino acid sequence of the 39 kDa antigen of the UP2 fraction of T. hyo.; however, they have common epitopes recognized in a single sera. As hereinafter indicated, the 39 kDa recombinant produced in this Example corresponds to a protein encoded by gene 1, one of the multiple genes encoding different T. hyo antigens, each having a molecular weight of about 39kDa.

As discussed in Example 4, infra, the T. hyo genome contains at least 7 genes encoding related antigens with molecular weights of about 39 kDa. Although the product of only one of these genes is isolated from cells grown in vitro, it is possible that the other members of the gene family are expressed in vivo and are of immunological relevance to protecting against infection in the field. The observation that each of these proteins is preceded by a signal sequence indicates that they will all be exported from the cytoplasm of the cell when expressed. When cells grown in vitro are surface labeled in the presence of I^{125} and lactoperoxidase the 39kDA protein in the KGP fraction is the predominant protein identified. Thus, cells expressing other members of the 39kDa gene family would likely have a much different surface architecture than cells expressing the Copy5 gene in vitro. An immune response mounted against one form of the 39kDa gene family could be only marginally effective against cells expressing one of the other forms.

Example 5

Identification of the genes encoding additional members of the 39 kDa antigen family

Internal amino acid sequence from 39p was divergent enough from the predicted amino acid sequence of pTrep301 to allow the selection and synthesis of a degenerate oligonucleotide (Cod664, Table 2) that could be used to distinguish between sequences encoding the gene product of pTrep 106 and those encoding the 39 kDa antigen.

The lambda GT11/ B204 library used in the pTrep 106 screening was probed with Cod664 as well as a nick translated probe made from a 411 base pair SphI-BclI fragment encoding the amino terminal portion of the 39kDa protein from pTrep301.

In addition a library constructed by the ligation of a partial Sau3a digest of B204 genomic DNA and BamHI digested lambda EMBL3 was also screened with these probes. This screening identified a number of phages which hybridized to either Cod664, the nick translated probe 301 Sph-Bcl or both probes.

Hybridizing phage were purified and subcloned into pUC8, 9, 18 or 19 for sequencing and additional manipulations for expression. The recombinant lambda phage, their hybridization patterns, and subclones are elaborated in Table 3 following the Examples.

Based on the DNA sequence from the above subclones and the internal peptide sequences, it was determined that there were at least six related genes encoding similar 39 kDa proteins. Of these six genes, analysis of protein and nucleotide sequence data indicate that gene #5 most likely encodes the 39kDa antigen found in the UP-2 and 39* fractions.

None of the subclones or combinations of subclones contained a full length copy of the #5 gene. Therefore, additional probes were prepared to isolate the remaining portion of the gene encoding the 39kDa antigen of the UP-2 and 39* fractions as well as other genes of the 39kDa family. These probes were based on additional internal sequences of the native antigen as well as DNA sequences of phages of Table 3.

A unique oligonucleotide probe specific for Gene #5 (Cod968) (Table 2) was synthesized and used to screen the GT11 library. Two degenerate oligonucleotides (Cod 1019 and Cod 1020) derived from peptide sequence of a carboxy terminal fragment from the 39s and 39p antigens were also used to screen the GT11 library to obtain a phage(s) which contained more extended coding sequences for the #5 gene. The

degeneracy of Cod 1019 and 1020 was decreased by assuming that codon usage for some amino acids would be similar to that found in other genes in the 39 kDa family.

Unique oligonucleotides were also synthesized and used to screen the GT11 library for full length genes corresponding to Gene #6 (Cod 934), Gene #7 (Cod 1010 and Cod 1011) and Gene #8 (Cod 1328).

A summary of all of the phages identified through this additional screening, their hybridization patterns and subclones is contained in Table 4.

DNA sequence derived from overlapping sublcones indicates that the 39 kDa genes are found in two subfamilies of tandemly repeated 39kDa genes. Family 1 contains (1-4) and Family 2 contains (5-8). Each gene encodes a protein with a presumptive signal sequence directing transport of the protein through the inner bacterial membrane.

The gene sequence and predicted amino acid sequence for each of the full length genes 1-8 is shown in Appendices 2 and 2A. In addition, Figure 1 of the drawings is a map of the gene family and sub-clones obtained from screening the two libraries.

Appendix 1 shows the relationship between the predicted amino acid sequences of processed products of genes 1-7 encoding seven different full length 39 kDa T. hyo antigens as well as the carboxy terminal fragment of gene #8.

The Perkin-Elmer/Cetus polymerase chain reaction system was used as a supplement to screening phage libraries to identify clones containing full length copies of 39 kDa genes. DNA sequence of Genes #1, 2, 3 (only 3') and 4 (only 5') indicated that these genes, although containing unique internal DNA

sequences, contained identical 5' and 3' DNA sequences. Thus, two linkered oligonucleotides corresponding to the 5' sequence (Cod 987), and the reverse complement of the 3' sequence (Cod 988), were synthesized to be used as primers for DNA synthesis. They were then mixed with a template of genomic DNA from either serotype B204 or B234. The oligonucleotide/DNA mixtures were passed through 25 cycles of heat denaturation, annealling and Taq polymerase directed DNA synthesis to amplify genomic DNA sequences between the two oligonucleotide primers. The newly synthesized amplified sequences were digested with Bam HI and Hind III, and cloned into pUC8 or pUC9. If cloned into pUC8 the fragments were oriented in the proper direction and in the proper reading frame for expression from the Lac promoter of a fusion protein comprised of 9 amino acids encoded by the pUC polylinker followed by a full-length copy of the mature forms of the corresponding antigens. Clones were initially screened by the Dot Blot Screening Protocol with unique and discriminating synthetic oligonucleotides derived from clones containing the full-length sequence of Gene #1 (Cod 844), or Gene #2 (Cod 931), or the partial sequence of Gene #3 (Cod 908), and Gene #4 (Cod 932, 1151). In addition the clones were screened with a unique synthetic oligonucleotide which is common to all known forms of the 39kDa gene family (Cod 957). Some clones hybridized only to this nondiscriminant probe and upon DNA sequence analysis were found to correspond to Gene #7 even though there is a slight mismatch of DNA sequence between Cod 987 and the 5' and 3' ends of the gene. A summary of the subclones obtained along with their

hydridization patterns is found in Table 4 following the Examples.

The PCR system was also used to synthesize and clone the #5 gene encoding the full length 39p/39s antigen. The oligomers used in this procedure were Cod 1054 and Cod 1055. Cod 1054 was derived from the DNA sequence of the 5' end of gene #6, a gene encoding a protein whose amino terminal amino acid sequence is identical to that of the 39s/39p antigen. Cod1055 was derived from the reverse complement of the DNA sequence encoding the carboxy terminal peptide of the 39s/39p antigen. This sequence distinguishes the #5 gene from any other gene obtained to date. The oligonucleotides were then mixed with genomic DNA from either B204 or B234 and passed through 25 cycles of heat denaturation, annealling and DNA synthesis in the presence of Taq polymerase in order to amplify intervening sequences. The amplified mixture was digested with BamHl and Hind3 and cloned into either pUC8 or 9. Candidate clones were screened for hybridization to Cod968, Cod1019, Cod 1020 and Cod 957. One of those clones, pTrep 613, includes the entire coding sequence for gene which is expressed under control of the beta-galactosidase promoter of pUC.

The PCR system was also used to synthesize and clone the #8 gene encoding the full length Copy 8 Antigen. The oligomers used in this procedure were Cod 1359 and Cod 1438, corresponding to the 3' and 5' ends of the gene, respectively. The oligonucleotides were mixed with genomic DNA from either B204 or B234 and passed through 25 cycles of heat denaturation, annealing, and DNA synthesis in the presence of Taq polymerase in order to amplify intervening sequences. The amplified mixture was digested with BamHI and

SalI and cloned into either pUC8 or 9. Candidate clones were screened for hybridization to Cod 957. One of these clones, pTrep 541, includes the entire coding sequence for the gene which is expressed under control of the beta-galactosidase promoter of pUC.

A schematic of the B204 expression clones derived from the PCR reaction (pTrep 345, 541, 604, 605, 620, 613) is found in Figure 5. The predicted amino acid sequences encoded by these clones are found in Appendix 5.

Expression of recombinant forms of the 39 kDa protein from genomic DNA subclones corresponding to Genes #2 and 6.

pTrep505, a pUC19 based plasmid directs the expression of all but the first 19 amino acids of Gene #2 of the 39 kDa gene family from the Lac promoter. It was constructed from pTrep 323 which contained an EcoR1 fragment subcloned from the lambda GT11 library. This EcoR1 fragment was subcloned into pWHA142 to place it in the proper orientation and reading frame for expression from the Lac promoter. pWHA142 is a derivative of pUC19 with a GAA reading frame across the EcoR1 site. A plasmid map of pTrep505 is presented in Figure 2. The predicted protein sequence from pTrep 505 is presented in Appendix 8.

pTrep 702, a pUC19 based plasmid directs the expression of 13 amino acids from the signal sequence of Gene #6 plus the first 315 amino acids of the mature protein fused to the LacZ complementing peptide in pUC. It was constructed in two steps from pTrep501 and pTrep327 which contain overlapping regions of the #6 gene and share a common Bcl1 site. pTrep501, which contains regions coding for the 5'

portion of the #6 gene, was digested with Bcll and Aatll and ligated with Bcll-Aatll fragment from pTrep 327 which contained the 3' sequences of the #6 gene. The EcoRl fragment from this clone, pTrep701, was then cloned into the EcoRl site of pWHA142 to place the #6 sequence in the proper reading frame for expression from the Lac promoter. A schematic of the construction of pTrep702 is presented in Figure 3. The predicted protein sequence of the recombinant product encoded by pTrep702 is presented in Appendix 6.

An expression clone encoding the full length Copy #6 antigen, pTrep704, was constructed from pTrep702 by replacing its 430 bp Nsil-Ndel fragment with an 847 bp Nsil-Ndel fragment from pTrep508. The 3' cloning site of pTrep508 is downstream of the cloning site contained within pTrep702 and thus contains the DNA sequences encoding the carboxy terminal portion and stop codon of the Copy #6 antigen which are lacking in pTrep702. A schematic of the construction of pTrep704 is presented in Figure 3. The predicted protein sequence of the recombinant product encoded by pTrep704 is presented in Appendix 7.

The recombinant products expressed by pTrep505, pTrep702, and pTrep704 as well as the PCR derived constructs are recovered as insoluble cements in E.coli strain CY15000 after lysis of cells with lysozyme and extraction with Triton X-100 in the presence of EDTA.

These are immunoreactive with sera from animals experimentally infected with T.hyo (B204) and with sera from animals vaccinated with UP2 or the electroeluted 39 kDa protein from UP2.

The following Table 5 tabulates the 39 kDa expression clones for expressing seven different T. hyo. antigens having a molecular weight of about 39 kDa, with reference to the different genes or copies.

Table 1 - Amino Acid Comparison of 39s and 39p Antigens

Sequence	Source
MYGDRDSWIDFLTHGNQFRARMDQLGFVLGN?TIKGTF??R	39p Nterm
MYGDRDSWIDFLTHGNQFRARMDQLGFVL?NGTIKGTFGF??Q?I	39* Nterm
P?S??TK?YMGISTDIQLRYYTGIDAFNAIRLYFKYGQAGFK	39p[2,4]
FPYS?STKDYMGISTDIQLRYYTGIDAFNAIRLYFKYGQAGFK	39*[2]
TANGASEYFAQSLGFEARFYFLNTPVGNVTINPFIKVVNTA	39p[2]
TANGASEYFAQSLGFEARFYFLNTPVGNVTINPFIKVVNTAL	39*[2]
AQAVLGITANSDVVSLYVEPSLGYQATYLGK	39p[2]
AQAVLGITANSDVVSLYVEPSL	39*
HISENPYLNIDSK	39p[1]
HISENPYLNIDSK	39*[2]
VQHSLAWGAYAELYVRPVQDLE?YFEMDIN	39p[1]
RNGVPVNFATSTGIT?YLPALGG?Q	39p[2]
MDINNSDSKRNGVPVNFATSTGITWYLPALGGAQ	39*[3]

Notes--All sequence derived from fragments generated by digestion with LysC in the presence of SDS unless otherwise indicated.
[1]LysC digest in absence of SDS
[2]Fraction gave muplitple sequences which were resolved on basi of intensity and DNA predicted amino acid sequence
[3]GluC digest in presence of SDS
[4]Sequence begins with residue #2 due to machine failure and loss of residue #1

Single amino acid code used above is as follows:

A=ala	H=his	P=pro	W=trp
C=cys	I=Ile	Q=gln	Y=tyr
D=asp	K=lys	R=arg	
E=glu	L=leu	S=ser	
F=phe	M=met	T=thr	
G=gly	N=asn	V=val	

Table 2

COD	Sequence	Source of Sequence	Specifi
664	ACG-AAG-GAT-TAT-ATG-GG A A C C T C	39p internal peptide sequence	for Copy 5,
844	TTAATCCGCATGATA	pTrep 106	
908	GTTTCATCACAAGCAAA	pTrep 333	3,
931	ATGAATATGACGGATAA	pTrep 330	
932	AAAGTTGATAAACAAGG	pTrep 333	
934	TATCATCCTTCTAATCCT	pTrep 331	
957	CCGAAAGTACCTTTAAT	pTrep 106	ALI
968	TATAATCCTTATGATCCT	pTrep 317	
987	GAATTCCGGATCCATGTATGG- AGATCAGGACGATTGGATT	pTrep 106	1-
988	GTCGACAAGCTTATAATTAAA- ATTCTGGCAAATACCAAGT	pTrep 106	1-
1010	ATATTGACTGATAGTAT	pTrep 506	
1111	AAATAATTTTGATATG	pTrep 506	
1020	GAT-AGA-AAA-AGG-AAT-GG TCT A C C	39* internal peptide sequence	
1019	AAA-AGG-AAT-GGA-GTG-CC A C T A T C	39* internal peptide sequence	
1054	GAATTCCGGATCCATGTATGGCGACAG- AGATTCTTGGATC	pTrep 326	5,
1055	GTCGACAAGCTTATAATTATTGAGCAC- CGCCTAAAGCAGG	pTrep 510	
1092	AGTATGTTTGAACCAATA	pTrep 333	
1151	TCATATGTATCGTGTATA	pTrep 333	
1095	GGAGTACCTAAACTTCAA	pTrep 337	
1248	CGACAGAGATTCTTGGA	pTrep 613	5,

Table 2 (continued)

COD	Sequence	Source of Sequence	Specifi- for Copy #
1328	GAATTCAATTACGGATT	pTrep 537	n
1359	GTCGACCTGCAGTTATTA-TTGTAAAGCAGGTAAATA-CCA	pTrep 520	R
1438	GAATTCCGGATCCATGTATGG-TGCAGACAACACATGGCTT	pTrep 537	R

Table #3

Table #3--Identification of recombinant phage and subclones

		Identified by Screening with	
Phage*	pTrep	301Sph-BclI	Cod664
9B^	317		+
54-3	323	+	
53-3B	326	+	+
52-1A	327	+	+
55-1	329	+	
56-3	330	+	
51-3	333	+	
51-1B	331	+	+
53-1B	337	+	+

* All recombinant phage are from the GT11 library unless otherwise noted
^ From EMBL3 library

Table #4

Table #4--Identification of recombinant phage and subclones

		Identified by Screening with												
Phage*	pTrep	301Sph-Bcl	908	934	968	1010	1019	1020	1092	1095	1151	1248	1326	957
21	506	+		+										
17	509	+		+										
130	517	+				+								
544	525		+						+					
163	510						+	+						
2	631										+			
519	520									+				
109	515				+									
441	349					+						+		
101A	537												+	+

Table 5
Expression Clones from PCR

Clone	Serotype	Cod857	Cod844	Cod908	Cod931	Cod932	Cod968	Cod1151
pTrep345	B204	+						+
pTrep605	B204	+			+			
pTrep604	B204	+		+		+		
pTrep608	B234	+			+			
pTrep609	B234	+				+		
pTrep610	B234	+	+					
pTrep613	B204	+					+	
pTrep620	B204	+						
pTrep651	B234	+						
pTrep541	B204	+						

Table 6

Clone	Copy#	Source	Comments
pTrep301	1	genomic	
pTrep505	2	genomic	lacks amino terminus
pTrep604	3	pcr	
pTrep345	4	pcr	
pTrep613	5	pcr	
pTrep702	6	genomic	lacks carboxy terminus
pTrep704	6	genomic	full length
pTrep620	7	pcr	1 amino acid substitution at C terminus relative to genomic sequence
pTrep605	2	pcr	full length
pTrep541	8	pcr	full length

Example 6

Use of 39* Antigen in a Vaccine

In two vaccination studies, purified 39* which includes 39s antigen and which is prepared according to the procedure of Example 1 (note pages 11 and 12) was tested in comparison with no challenge, vaccination with adjuvant, or vaccination with the commercially available Hyguard (Haver Labs) product.

In the first study, six pigs per test group were used. The pigs averaged 22.6 lb and were approximately 5-6 weeks of age. Five groups of pigs were given two doses, the first on day 0 and a booster on day 36. The injections were given I.M., in the neck with 1 mg/dose of native antigen. Animals were challenged on day 50 by stomach intubation using a pure culture of T. hyo. (B204) at 5.5×10^2 cfu per pig. The study was terminated on day 92.

Vaccines were given with Emulsigen adjuvant. Emulsigen was used as an adjuvant control, mixed with Dulbeco's PBS buffer. The Hyguard, a bacterin, was adminstered according to manufacturer's directions.

Animals were monitored daily for clinical signs of swine dysentery. Microbiological evaluation of routine weekly rectal swabs was conducted for T. hyo. and Salmonella. Animals showing signs of bloody diarrhea were swabbed and evaluated on that day. Weekly postchallenge pigs were weighed and their feed intake determined. The experimental results are shown below.

Overall summary comparison of treatment groups during the 42-day postchallenge period

Treatment	% Pig Days of Normal: Anorexia	% Pig Days of Normal: Reduction	% Pig Days of Normal: Fever	Diarrhea: % Pig Days	Diarrhea: No. of Pigs	Diarrhea: Mean No. Days to Onset	ADG (lb)	Feed-to-Gain	Mean # Recovery of T. hyo
No Challenge	100	99	99	0	0	N/A	1.87	2.76	0
Adj + Buffer	96	78	84	8	4	18	1.35	3.33	19
Hydrated	100	79	96	0	0	N/A	1.50	2.98	3
39*	100	84	100	0	0	N/A	1.78	2.76	13

Key: ADG; average daily weight gain in pounds

Mean & Recovery of T. hyo.; percentage of samples from which T. hyo. parasite was recovered from rectal swabls of challenged animals.

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Example 7

Use of Native Antigen

A second study was conducted to confirm the results of Example 5 where the 39* vaccinates performed exceptionally well when challenged intra-gastrically with a measured dose of T. hyodysenteriae. As shown below, 1 of 9 animals vaccinated with a 1 mg dose of 39* and challenged by stomach intubation with 4.4×10^2 CFU of T. hyo (B204) developed clinical signs of swine disentery in comparison to 3 of 6 adjuvant vaccinates and 0 of 6 HyGuard vaccinates.

Vaccinate Group	# of Breaks/ # Animals	Day of Onset
No Challenge	0/6	
Adjuvant	3/6	11, 11, 12
HyGuard	0/5	
39*, 1mg	1/6	29
39*, 1mg--new lot	0/3	

Example 8

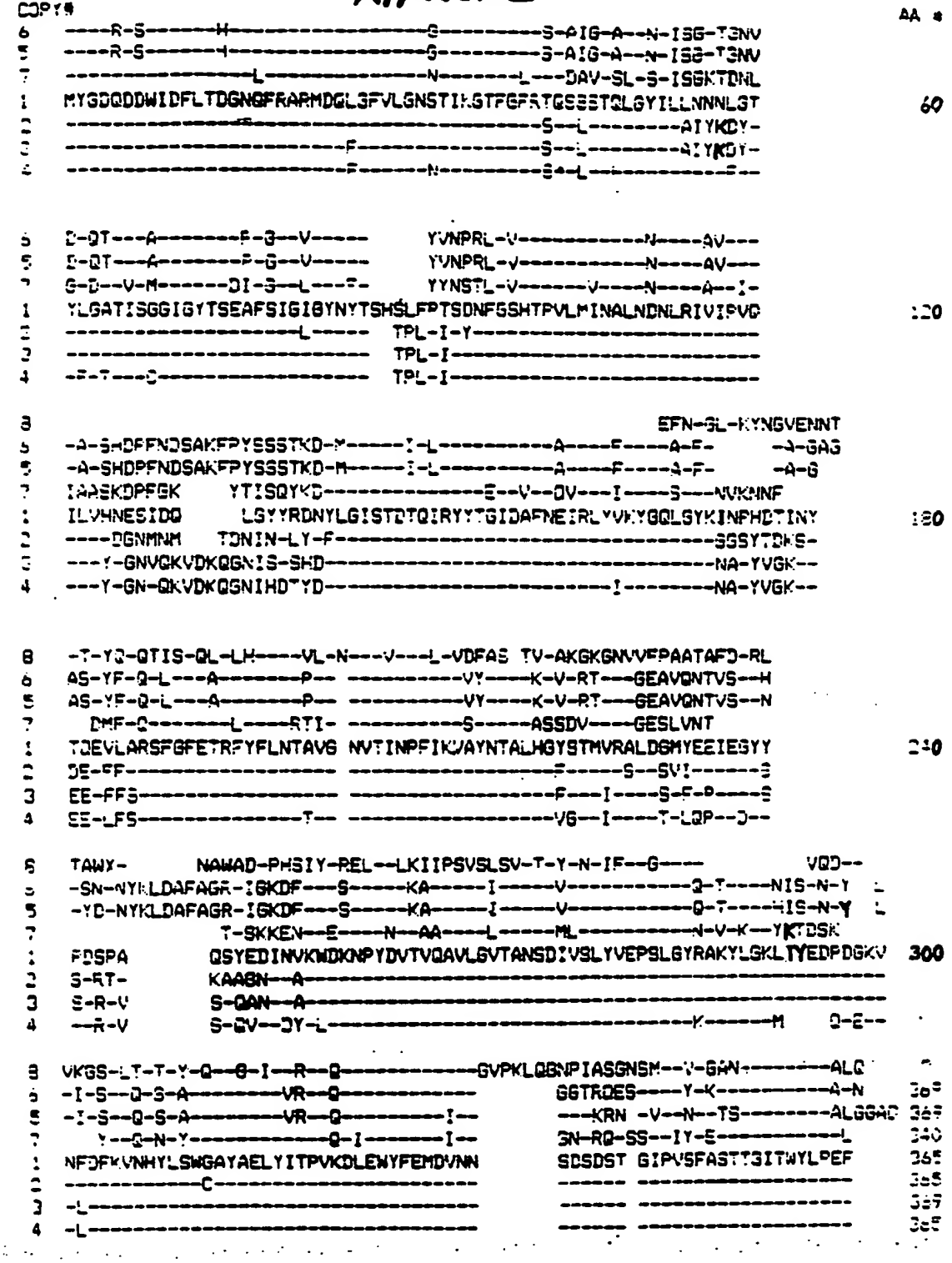
Production of Antibody

A cement preparation (Part G of Example 4) in an amount of 25-200 µg in Emulsigen is intermuscularly injected into pigs. Two weeks later, the pig is boosted with an identical dose. Two weeks after the boost, the pig is bled and the blood is allowed to clot. Immune serum is separated by centrifugation at 4°C.

Numerous modifications and variations of the present invention are possible in light of the above teachnings; therefore, within the scope of the

appended claims, the invention may be practiced otherwise than as particularly described.

Appendix 1



Appendix 2A

FIGURE # --DNA Sequence of B204 Genes Encoding 39 kDa Antigens 1-4

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   1  GAATTCTATCAGAATATTTTTTTATAATATAAATATTTTTTTTATCTTCTATATCTATA

  60 TATTTGCTGAGAT4ATCTTTTAATACATAATAANGCATTTTTTTNCATTNATTTTCANTT

 120 TGTTAAATCATATATAATATTGTTAATATCATTAATTAAATTATCAATATTTAAATTATT

 180 ACTATCTTGTTTTTTTATTTTTTATCATAGAT9AAGCACCAGCAGCTCCTATAGCCAATG

 240 GTATACCTATCACAGGCAATAAGGTAGCTCCTAATACAGCACTAGTTGCTGCTGCCGTTG

 300 CACCTATAGCACCAGCTGCTACTGCAGTACCGCCTAATAAACTAGAATATAAAGATGGTA

 360 TTTGTTTTATTGATTCATTATTTTTAGATAATTCTTGCATCAAATATGTTAAGTTTTTTA

 420 AAATAGGTATGAATTTTGATGAATATATTTCTTCTTCTATATTTTTTGTGCTATTAAAAC

 480 AAGAAAATAAAATTTTTCCTATTGTGCTTTTTCCAGTGTTATTCTCTCCGGCTATTACAG

 540 TAATACCATCTATTGTTATATTAGCTTCTTTGATTTTAGAAAAATTTTTTATATTCAATT

 600 CCATTATATTTTATCCTTTAATAATTCTGTTATTATTATAACATAAAAAATATTTAATAA

 660 AAATATATTAATTTTMATTAAATCTAATTCTTGAGCTATTTTATATTTTTAGTATAATAA

                                        * Presumed translational start
                                      MetLysLysPhePheLeuIleMetThrVal
 720 AAAATATAAACTTCAATTTAGGTATGTATAATGAAAAAGTTTTTTCTAATTATGACAGTA

      of Copy 1
     LeuLeuSerMetSerTyrCysSerIlePheGlyMetTyrGlyAspGlnAspAspTrpIle
 780 TTATTAAGTATGTCATATTGTTCAATTTTTGGTATGTATGGAGATCAGGACGATTGGATT

     AspPheLeuThrAspGlyAsnGlnPheArgAlaArgMetAspGlnLeuGlyPheValLeu
 840 GATTTTCTTACAGACGGCAATCAGTTTAGAGCTAGAATGGATCAATTAGGATTTGTTTTA

     GlyAsnSerThrIleLysGlyThrPheGlyPheArgThrGlnSerSerSerThrGlnLeu
 900 GGTAATAGTACTATTAAAGGTACTTTCGGTTTTAGAACTCAAAGTTCATCAACTCAATTA

     GlyTyrIleLeuLeuAsnAsnAsnLeuGlyThrTyrLeuGlyAlaThrIleSerGlyGly
 960 GGATATATTTTGTTGAATAATAATCTTGGTACTTATTTGGGAGCAACTATTTCTGGCGGT

     IleGlyTyrThrSerGluAlaPheSerIleGlyIleGlyTyrAsnTyrThrSerHisSer
1020 ATAGGATATACTTCTGAGGCTTTTAGTATAGGCATAGGCTATAATTATACCAGCCATTCC

     LeuPheProThrSerAspAsnPheGlySerHisThrProValLeuMetIleAsnAlaLeu
1080 TTATTTCCTACTAGCGATAACTTTGGTTCTCATACTCCAGTACTTATGATTAATGCTTTA

     AsnAspAsnLeuArgIleValIleProValGlnIleLeuValHisAsnGluSerIleAsp
1140 AATGATAATTTGAGGATAGTTATTCCTGTGCAAATATTAGTACATAATGAAAGTATTGAT

     GlnLeuGlyTyrTyrArgAspAsnTyrLeuGlyIleSerThrAspThrGlnIleArgTyr
1200 CAACTTGGTTACTATAGAGATAATTATTTAGGTATAAGTACTGATACGCAAATAAGATAT

     TyrThrGlyIleAspAlaPheAsnGluIleArgLeuTyrValLysTyrGlyGlnLeuGly
1260 TATACAGGCATAGATGCTTTTAATGAAATAAGATTATATGTAAAATATGGGCAATTAGGA
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     TyrLysIleAsnProHisAspThrIleAsnTyrThrGlnGluValLeuAlaArgSerPhe
1320 TATAAAATTAATCCGCATGATACTATAAATTATACACAAGAAGTTTTAGCAAGATCATTT

     GlyPheGluThrArgPheTyrPheLeuAsnThrAlaValGlyAsnValThrIleAsnPro
1380 GGTTTTGAAACAAGATTCTATTTTTTGAATACTGCTGTTGGAAATGTAACTATCAATCCT

     PheIleLysValAlaTyrAsnThrAlaLeuHisGlyTyrSerThrMetValArgAlaLeu
1440 TTTATTAAAGTAGCATATAATACAGCTTTGCATGGATATAGTACCATGGTAAGAGCATTG

     AspGlyMetTyrGluGluIleGluGlyTyrTyrProAspSerProAlaGlnSerTyrGlu
1500 GATGGTATGTATGAAGAAATAGAAGGTTATTATCCAGATAGTCCTGCTCAATCATATGAA

     AspIleAsnValLysTrpAspLysAsnProTyrAspValThrValGlnAlaValLeuGly
1560 GATATTAATGTTAAATGGGATAAGAATCCTTATGATGTAACTGTGCAGGCAGTATTGGGA

     ValThrAlaAsnSerAspIleValSerLeuTyrValGluProSerLeuGlyTyrArgAla
1620 GTAACTGCTAATAGCGATATAGTATCACTTTATGTTGAGCCTTCTTTAGGTTATAGGGCT

     LysTyrLeuGlyLysLeuThrTyrGluAspProAspGlyLysValAsnPheAspPheLys
1680 AAATATTTAGGAAAATTAACATATGAAGATCCAGATGGAAAAGTTAATTTTGATTTTAAA

     ValAsnHisTyrLeuSerTrpGlyAlaTyrAlaGluLeuTyrIleThrProValLysAsp
1740 GTTAATCATTATTTATCTTGGGGTGCTTATGCAGAGCTTTATATAACACCGGTAAAAGAT

     LeuGluTrpTyrPheGluMetAspValAsnAsnSerAspSerAspSerThrGlyIlePro
1800 TTAGAATGGTATTTTGAAATGGATGTTAATAATAGTGATTCAGATTCTACAGGTATACCT

     ValSerPheAlaSerThrThrGlyIleThrTrpTyrLeuProGluPheOC
1860 GTTAGTTTTGCTTCTACTACAGGAATAACTTGGTATTTGCCAGAATTTTAATTATAAAGC

1920 AAATTTTATATGATAAAATAAAAAATGTGGGGTATTTATTATTAAAAAATAAATACCCCA

1980 CATTTTATTAAATAATTTTTTCAGTAATTTTACATTTATATATTTTTTAGTATAATAAAA

                                        * Presumed translational start of
                                       MetLysLysIlePheLeuIleMetThrValLeu
2040 ATATAAACTTAAATTTAGGTATATACAATGAAAAAAATTTTTCTAATTATGACAGTATTA

     Copy 2
     LeuSerMetSerTyrCysSerIlePheGlyMetTyrGlyAspGlnAspAspTrpIleAsp
2100 TTAAGTATGTCATATTGTTCAATATTTGGTATGTATGGAGATCAGGACGATTGGATTGAT

     PheLeuThrAspGlyAsnGlnPheArgAlaArgMetAspGlnLeuGlyPheValLeuGly
2160 TTTCTTACAGACGGCAATCAGTTTAGAGCTAGAATGGATCAATTAGGATTTGTTTTAGGT

     AsnSerThrIleLysGlyThrPheGlyPheArgSerGlnSerLeuSerThrGlnLeuGly
2220 AATAGCACCATTAAAGGTACTTTCGGTTTTAGATCTCAGAGTTTATCAACTCAATTAGGA

     TyrIleLeuAlaIleTyrLysAspTyrThrTyrLeuGlyAlaThrIleSerGlyGlyIle
2280 TATATTTTGGCTATATATAAAGATTATACTTATTTAGGAGCAACTATTTCCGGCGGTATA

     GlyTyrThrSerGluAlaPheSerIleGlyLeuGlyTyrAsnTyrThrThrProLeuPro
2340 GGATATACTTCTGAGGCTTTTAGTATAGGTTTAGGTTATAATTATACTACACCGCTTCCT

     IleSerTyrAsnPheGlySerHisThrProValLeuMetIleAsnAlaLeuAsnAspAsn
2400 ATTAGTTATAACTTTGGTTCTCATACTCCTGTACTTATGATTAATGCTTTAAATGATAAT
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     LeuArgIleValIleProValGlnIleLeuValHisAspGlyAsnMetAsnMetThrAsp
2460 TTGAGGATAGTTATTCCTGTACAAATATTAGTACATGATGGTAATATGAATATGACGGAT

     AsnIleAsnTyrLeuTyrAsnPheLeuGlyIleSerThrAspThrGlnIleArgTyrTyr
2520 AATATTAATTATTTATATAATTTTTTAGGTATAAGTACTGATACTCAAATAAGATATTAT

     ThrGlyIleAspAlaPheAsnGluIleArgLeuTyrValLysTyrGlyGlnLeuGlyTyr
2580 ACAGGCATAGACGCTTTTAATGAAATAAGATTATATGTAAAATACGGACAATTAGGATAT

     LysGlyGlySerTyrThrAspLysSerTyrAspGluGluPhePheAlaArgSerPheGly
2640 AAAGGCGGTTCATATACGGATAAAAGTTATGATGAAGAATTTTTTGCAAGATCATTTGGT

     PheGluThrArgPheTyrPheLeuAsnThrAlaValGlyAsnValThrIleAsnProPhe
2700 TTTGAAACAAGATTCTATTTTTTGAATACTGCTGTTGGAAATGTAACTATCAATCCTTTT

     IleLysValAlaTyrAsnThrAlaLeuHisGlyPheSerThrMetValArgSerLeuAsp
2760 ATTAAAGTAGCATATAATACAGCTTTGCATGGATTTAGTACTATGGTAAGATCATTAGAT

     SerValIleGluGluIleGluGlyTyrSerSerAspArgThrAlaLysAlaAlaGlyAsn
2820 AGTGTCATTGAAGAAATAGAAGGTTATAGTTCAGATCGTACCGCTAAAGCAGCAGGAAAT

     IleAsnAlaLysTrpAspLysAsnProTyrAspValThrValGlnAlaValLeuGlyVal
2880 ATTAATGCTAAATGGGATAAGAATCCTTATGATGTAACTGTGCAGGCAGTATTGGGAGTA

     ThrAlaAsnSerAspIleValSerLeuTyrValGluProSerLeuGlyTyrArgAlaLys
2940 ACTGCTAATAGCGATATAGTATCACTTTATGTTGAGCCTTCTTTAGGTTATAGGGCTAAA

     TyrLeuGlyLysLeuThrTyrGluAspProAspGlyLysValAsnPheAspPheLysVal
3000 TATTTAGGAAAATTAACATATGAAGATCCAGATGGAAAAGTTAATTTTGATTTTAAAGTT

     AsnHisTyrLeuSerTrpCysAlaTyrAlaGluLeuTyrIleThrProValLysAspLeu
3060 AATCATTATTTATCTTGGTGTGCTTATGCAGAGCTTTATATAACACCTGTAAAAGATTTA

     GluTrpTyrPheGluMetAspValAsnAsnSerAspSerAspSerThrGlyIleProVal
3120 GAATGGTATTTTGAAATGGATGTTAATAATAGTGATTCAGATTCTACAGGTATACCTGTT

     SerPheAlaSerThrThrGlyIleThrTrpTyrLeuProGluPheOC
3180 AGTTTTGCTTCTACTACAGGAATAACTTGGTATTTGCCAGAATTTTAATTATAAAGCAAA

3240 TTTTATATGACAAAATAAAAAATGGGGCATTTATTATTAAAAAATAAATACCCCACATTT

3300 TATTAAATAACTTCTTAAATAATTTTACA4TTTATATTTTATTAGTATAATAAAATATAA

                                   * Presumed translational start of Copy 3
                                  MetLysLysSerPheLeuIleMetThrValLeuLeuSer
3360 AGTTAAATTTAGGTGTGTACAATGAAAAAAAGTTTTCTAATTATGACAGTATTATTAAGT

     MetSerTyrCysSerIlePheGlyMetTyrGlyAspGlnAspAspTrpIleAspPheLeu
3420 ATGTCATATTGTTCAATATTTGGTATGTATGGAGATCAGGACGATTGGATTGATTTTCTT

     ThrAspGlyAsnGlnPheArgAlaArgMetAspGlnPheGlyPheValLeuGlyAsnSer
3480 ACAGACGGTAATCAGTTTAGAGCTAGAATGGATCAATTTGGATTCGTTTTAGGTAATAGC

     ThrIleLysGlyThrPheGlyPheArgSerGlnSerLeuSerThrGlnLeuGlyTyrIle
3540 ACCATTAAAGGTACTTTCGGTTTTAGATCTCAGAGTTTATCAACTCAATTAGGATATATT

     LeuAlaIleTyrLysAspTyrThrTyrLeuGlyAlaThrIleSerGlyGlyIleGlyTyr
3600 TTGGCTATATATAAAGATTATACTTATTTAGGAGCAACTATTTCCGGCGGTATAGGATAT
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ThrSerGluAlaPheSerIleGlyLeuGlyTyrAsnTyrThrThrProLeuProIleSer
3660 ACTTCTGAGGCTTTTAGTATAGGTTTAGGTTATAATTATACTACACCGCTTCCTATTAGT

AspAsnPheGlySerHisThrProValLeuMetIleAsnAlaLeuAsnAspAsnLeuArg
3720 GATAACTTTGGTTCTCATACTCCTGTACTTATGATTAATGCTTTAAATGATAATTTGAGG

IleValIleProValGlnIleLeuValTyrAsnGlyAsnValGlnLysValAspLysGln
3780 ATAGTTATTCCTGTACAAATATTAGTATATAATGGTAATGTTCAAAAAGTTGATAAACAA

GlyAsnIleSerTyrSerHisAspTyrLeuGlyIleSerThrAspThrGlnIleArgTyr
3840 GGTAATATCTCTTATTCACATGATTATTTAGGTATAAGTACTGATACGCAAATAAGATAT

TyrThrGlyIleAspAlaPheAsnGluIleArgLeuTyrValLysTyrGlyGlnLeuGly
3900 TATACAGGTATAGATGCTTTTAATGAAATAAGATTATATGTAAAATATGGGCAATTAGGA

TyrLysAsnAlaProTyrValGlyLysAsnTyrGluGluGluPhePheSerArgSerPhe
3960 TATAAAAATGCTCCGTATGTTGGTAAGAATTATGAAGAAGAATTTTTTTCAAGGTCATTT

GlyPheGluThrArgPheTyrPheLeuAsnThrAlaValGlyAsnValThrIleAsnPro
4020 GGTTTTGAAACAAGATTCTATTTTTTGAATACTGCTGTTGGAAATGTAACTATCAATCCT

PheIleLysValAlaTyrAsnThrAlaLeuHisGlyPheSerThrMetIleArgAlaLeu
4080 TTTATTAAAGTAGCATATAATACAGCTTTGCATGGATTTAGTACCATGATAAGAGCATTA

AspSerMetPheGluProIleGluGlyTyrSerSerAspArgProValSerSerGlnAla
4140 GATAGTATGTTTGAACCAATAGAAGGTTATAGTTCAGATCGTCCTGTTTCATCACAAGCA

AsnIleAsnAlaLysTrpAspLysAsnProTyrAspValThrValGlnAlaValLeuGly
4200 AATATTAATGCTAAATGGGATAAGAATCCTTATGATGTAACAGTGCAGGCAGTATTGGGA

ValThrAlaAsnSerAspIleValSerLeuTyrValGluProSerLeuGlyTyrArgAla
4260 GTAACCGCTAATAGCGATATAGTATCACTTTATGTTGAGCCTTCTTTAGGTTATAGGGCT

LysTyrLeuGlyLysLeuThrTyrGluAspProAspGlyLysValAsnLeuAspPheLys
4320 AAATATTTAGGAAAATTAACATATGAAGATCCAGATGGAAAAGTTAATTTGGATTTTAAA

ValAsnHisTyrLeuSerTrpGlyAlaTyrAlaGluLeuTyrIleThrProValLysAsp
4380 GTTAATCATTATTTATCTTGGGGGGCTTATGCAGAGCTTTATATAACACCGGTAAAAGAT

LeuGluTrpTyrPheGluMetAspValAsnAsnSerAspSerAspSerThrGlyIlePro
4440 TTGGAATGGTATTTTGAAATGGATGTTAATAATAGTGATTCAGATTCTACAGGAATACCT

ValSerPheAlaSerThrThrGlyIleThrTrpTyrLeuProGluPheOC
4500 GTTAGTTTTGCTTCTACTACAGGAATAACTTGGTATTTGCCAGAATTTTAATTATAAAGC

4560 AAATTTTATATGACAAAATAAAAAATGNGGGNTATTTATTATTAAAAAATAAATACCCCG

4620 TTTTTTATTAAATAACTTCTTAAATAATTTTACATTTTTATATTTTATTAGTATAATAAA

* Presumed translational start of
MetLysLysPheLeuIleMetThrValLeuLeu
4680 ATATAAAGTTAAATTTAGGTGTGTACAATGAAAAAGTTTCTAATTATGACAGTATTATTA

Copy 4
SerMetSerTyrCysSerIlePheGlyMetTyrGlyAspGlnAspAspTrpIleAspPhe
4740 AGTATGTCATATTGTTCAATATTTGGTATGTATGGAGATCAGGACGATTGGATTGATTTT

LeuThrAspGlyAsnGlnPheArgAlaArgMetAspGlnPheGlyPheValLeuGlyAsn
4800 CTTACAGACGGTAATCAGTTTAGAGCTAGAATGGATCAATTTGGATTCGTTTTAGGTAAT

AsnThrIleLysGlyThrPheGlyPheArgSerGlnSerLeuSerThrHisLeuGlyTyr
4860 AACACCATTAAAGGTACTTTCGGTTTTAGATCTCAGAGTTTATCAACTCACTTAGGCTAT

IleLeuLeuAsnAsnAsnPheGlyThrTyrPheGlyThrThrIleSerCysGlyIleGly
4920 ATTTTGTTAAATAATAATTTTGGTACTTATTTTGGAACAACTATATCATGCGGTATAGGA

TyrThrSerGluAlaPheSerIleGlyIleGlyTyrAsnTyrThrThrProLeuProIle
4980 TATACTTCTGAGGCTTTTAGTATAGGTATAGGTTATAATTATACTACACCGCTTCCTATT

SerAspAsnPheGlySerHisThrProValLeuMetIleAsnAlaLeuAsnAspAsnLeu
5040 AGTGATAACTTTGGTTCTCATACTCCTGTACTTATGATTAATGCTTTAAATGATAATTTG

ArgIleValIleProValGlnIleLeuValTyrAsnGlyAsnIleGlnLysValAspLys
5100 AGGATAGTTATTCCTGTACAAATATTAGTATATAATGGTAATATTCAAAAAGTTGATAAA

GlnGlyAsnIleHisAspThrTyrAspTyrLeuGlyIleSerThrAspThrGlnIleArg
5160 CAAGGTAATATACACGATACATATGATTATTTAGGTATAAGTACTGATACGCAAATAAGA

TyrTyrThrGlyIleAspAlaPheAsnGluIleArgLeuTyrIleLysTyrGlyGlnLeu
5220 TATTATACAGGTATAGATGCTTTTAATGAAATAAGATTATATATAAAATATGGACAATTA

GlyTyrLysAsnAlaProTyrValGlyLysAsnTyrGluGluGluLeuPheSerArgSer
5280 GGATATAAAAATGCTCCGTATGTTGGTAAAAATTATGAAGAAGAACTTTTTTCAAGGTCA

PheGlyPheGluThrArgPheTyrPheLeuAsnThrThrValGlyAsnValThrIleAsn
5340 TTTGGTTTTGAAACAAGATTCTATTTTTTGAATACTACTGTTGGAAATGTAACTATTAAT

ProPheIleLysValAlaTyrAsnThrAlaLeuHisGlyValGlyThrMetIleArgAla
5400 CCTTTTATTAAAGTAGCATATAATACAGCTTTGCATGGAGTTGGTACTATGATAAGAGCA

LeuAspThrMetLeuGlnProIleGluAspTyrTyrProAspArgProValSerSerGln
5460 TTAGATACTATGCTTCAACCAATAGAAGATTATTATCCAGATCGTCCTGTTTCATCACAA

ValAspIleAspTyrLysLeuAspLysAsnProTyrAspValThrValGlnAlaValLeu
5520 GTAGATATTGATTATAAATTGGATAAGAATCCTTATGATGTAACTGTGCAGGCAGTATTG

GlyValThrAlaAsnSerAspIleValSerLeuTyrValGluProSerLeuGlyTyrLys
5580 GGAGTAACCGCTAATAGTGATATAGTATCACTTTATGTTGAGCCTTCTTTAGGTTATAAG

AlaLysTyrLeuGlyLysMetGlnAspGluLysValAsnLeuAspPheLysValAsnHis
5640 GCTAAATATTTAGGAAAAATGCAAGATGAAAAAGTTAATTTGGATTTTAAAGTTAATCAT

TyrLeuSerTrpGlyAlaTyrAlaGluLeuTyrIleThrProValLysAspLeuGluTrp
5700 TATTTATCTTGGGGTGCTTATGCAGAGCTTTATATAACACCTGTAAAAGATTTAGAATGG

TyrPheGluMetAspValAsnAsnSerAspSerAspSerThrGlyIleProValSerPhe
5760 TATTTTGAAATGGATGTTAATAATAGTGATTCAGATTCTACAGGTATACCTGTTAGTTTT

AlaSerThrThrGlyIleThrTrpTyrLeuProGluPheOC
5820 GCTTCTACTACAGGGATAACTTGGTATTTGCCAGAATTTTAATTATAAA6AAAATTTTAT

5880 ATGACAAAATAAAAAATAAGAGGTATTTATTTTTTAATAATAAATACCTCTCTTATTTT

5940 ATTTATA8TTTTTTAATTTAATCTCTTCTTCTTTGTGCTAACATTAAAAGTCTTAAAAGT

6000 GTAAGTATAGCAGTAACAGCAGCAGCAACATAAGTCAAAGCAGCAGCAGATAAAACTTTT

6060 TTAGCTCCGTCAAGTTCTTCACTGTCTAAAAATCCGCCTTTATCCAATATTTTTATAGCT

Appendix 2B

Figure # --DNA Sequence of B204 Genes Encoding 39 kDa Antigens 5-8

*5' end of genomic DNA

1 CTGTATCTGTTTATTATCAGTCAGCATTTCATTCTTATTATTATGGCGATAAAAATATAA

61 AAATGGAAACTATAGTCGGAAACTTAATAGGAAGCGT4GCCAAAGAAAATAAAGATGATA

121 TACCAAAATTAAAGAACTATTTTAATAATTCTGTAAAAATAAAAGCTGAGAAATTTGGTA

181 AAAAATGGAAAGACTATTATGAAAGCAGAAATCTTATAAATAATTTTAATTTATAATATA

241 TTACCGATAAAAACAGCATTAATGTTTTTTATAATACTGTATTTCACAAAAATATAGCAT

301 TATGTATTAAATAAACTTTAATATATCTTTATATTTTATACTTTTGACTGTAATATTAAA

361 TTATCATTTATATAACTATACCTAAATAGATATATTACTATAATTGTATCACAAATTAGA

421 TATAAACCATCATATTTAACATAAGAAAAAATATATTATTATTAAAATATAAAAATTTTT

481 GTATTTTTTATAGAATTTTAA9AAAAATTTTTATATAATAATATTCATATATATTAGGA4

* Presumed translational start of Copy 8

MetLys PheLeuLeuThrValLeuAlaIleLeuThrIleAlaSerGly

541 AAAATAAAAATGAAAAA6TTTTTATTAACAGTGCTGGCTATTTTAACAATAGCTAGCGGA

SerValPheGlyMetTyrGlyAlaAspAsnThrTrpLeuPhePheLeuIleHisGlyAsn

601 TCAGTGTTTGGTATGTATGGTGCAGACAACACATGGCTTTTCTTCCTCATACATGGCAAC

GlnPheArgAlaArgMetAsnGlnLeuGlyPheThrLeuGlyAsnGlyIleIleLysGly

661 CAATTCAGAGCTAGAATGAACCAATTAGGTTTCACTCTAGGTAACGGCATTATTAAAGGT

ThrPheGlyPheLysAlaAsnThrLeuIleAsnGlySerIleLeuAsnThrGlyAsnLys

721 ACTTTCGGTTTCAAAGCTAATACGCTTATTAACGGAAGCATCTTAAATACAGGCAATAAA

GluAsnGlnAsnProLeuGluAlaThrIleSerAlaGlyIleGlyTyrThrGlyAspGly

781 GAAAACCAAAATCCATTAGAAGCTACTATTTCTGCTGGTATAGGTTACACAGGTGATGGT

PheGlyValGlyValGlyTyrAsnTyrThrTyrThrAlaAlaAsnThrIleGlnThrLys

841 TTTGGTGTTGGTGTTGGTTATAACTATACTTATACTGCTGCAAATACTATTCAAACCAAA

AlaAlaLysGlyIleAsnThrHisThrProValIleThrPheAsnAlaValAsnAsnAsn

901 GCTGCTAAAGGAATAAATACTCATACACCTGTTATTACATTCAATGCTGTTAATAACAAT

LeuArgValAlaIleProValSerIleAlaValGluLysAspIleGlyLysLeuGlyAsn

961 TTAAGAGTAGCTATACCTGTAAGTATAGCTGTAGAAAAAGATATAGGTAAATTAGGTAAT

MetAspArgLysAspTyrLeuGlyLeuSerIleProAlaGlnIleArgTyrTyrThrGly

1021 ATGGATAGAAAAGATTATTTAGGTTTAAGCATACCTGCTCAAATAAGATATTATACAGGA

IleAspAlaPheAsnTyrIleArgPheGluPheAsnTyrGlyLeuAsnLysTyrAsnGly

1081 ATAGATGCTTTCAACTATATAAGATTTGAATTCAATTACGGATTAAATAAATATAATGGT

ValGluAsnAsnThrThrThrGluTyrGlnAlaGlnThrIleSerPheGlnLeuArgLeu

1141 GTTGAAAACAATACAACTACAGAATATCAAGCACAGACTATAAGCTTCCAATTAAGACTT

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      HisPheLeuAsnThrValLeuGlyAsnAsnValThrValAsnProPheLeuArgValAsp
1201  CATTTCTTAAATACAGTTCTTGGAAATAATGTAACTGTAAACCCATTCTTAAGAGTTGAT

      PheAlaSerThrValGlyAlaLysGlyLysGlyAsnValValPheProAlaAlaThrAla
1261  TTTGCTTCTACTGTAGGTGCTAAAGGAAAAGGAAATGTAGTATTCCCAGCAGCTACAGCT

      PheAspGlyArgLeuThrAlaTrp   AlaAsnAlaTrpAlaAspAspProHisSerIle
1321  TTTGATGGAAGACTTACAGCTTGG0CTGCAAATGCTTGGGCAGATGATCCACACAGCATT

      TyrAspArgGluLeuTyrAspLeuLysIleIleProSerValSerLeuSerValAsnThr
1381  TATGACAGAGAACTTTATGACTTGAAAATCATACCTAGCGTATCTTTAAGTGTTAATACA

      AspTyrValAsnLeuIlePheGluProGlyLeuGlyTyrArgValGlnAspAspGlyVal
1441  GACTATGTTAATTTAATATTTGAACCTGGTTTAGGATACAGAGTACAAGATGATGGTGTA

      LysGlySerLysLeuThrHisThrLeuTyrTrpGlnAlaTyrGlyGluIleTyrIleArg
1501  AAAGGAAGCAAACTTACTCATACATTATACTGGCAGGCTTACGGAGAAATATATATCAGA

      ProValGlnAspLeuGluTrpTyrPheGluMetAspValAsnAsnGlyValProLysLeu
1561  CCTGTTCAGGATCTTGAATGGTATTTCGAAATGGATGTTAATAACGGAGTACCTAAACTT

      GlnGlyAsnProIleAlaSerGlyAsnSerMetProValValPheGlyAlaAsnThrGly
1621  CAAGGAAATCCTATTGCTTCAGGAAATTCAATGCCTGTTGTATTCGGAGCTAATACTGGT

      IleThrTrpTyrLeuProAlaLeuGln
1681  ATAACTTGGTATTTACCTGCTTTACAATAATAAATAACAGTGAATAATCAGAAATAACTG

1741  ATTTTTAATACAAGGGGAGCTTCATTAAAAAGCTCCTCTTTTTTATGAGAAAAACAAATA

1801  TAAAGCTTNNGTTCATAATTATCAATTTAATCAAAACATACTAAGCAAATTCAAAATTTC

1861  ATAAAAAATAATTATTCAATAAAAATTCATAATTTTTTATTAAAAAAATATGYAAATATG

1921  TATTTGAATGACTATTTTTTTATATAATTCCTTTTATATGTATTGACATTTTATAACTTT

1981  TTCCTATAATGAAAGTAGAGGATCTTTAGGTGTCTATTGAGTTTTTAAGCGATCTCTCAA

                                                  * Presumed translational
                                                    MetLysLysValLeuLeu.
2041  TAGACACTGTCAAAAGATTATAAAAAAATTAGGAGAAAAACAATGAAAAAAGTTTTATTG

      start of Copy 6
      ThrAlaMetAlaLeuLeuThrIleAlaSerAlaSerAlaPheGlyMetTyrGlyAspArg
2101  ACAGCTATGGCATTATTGACTATAGCTAGTGCATCTGCTTTCGGTATGTATGGCGACAGA

      AspSerTrpIleAspPheLeuThrHisGlyAsnGlnPheArgAlaArgMetAspGlnLeu
2161  GATTCTTGGATCGACTTCCTTACTCATGGTAATCAGTTCAGAGCTAGAATGGATCAATTA

      GlyPheValLeuGlyAsnGlyThrIleLysGlyThrPheGlyPheArgSerGlnAlaIle
2221  GGTTTCGTTTTAGGTAACGGTACTATTAAAGGTACTTTCGGTTTTAGATCTCAAGCTATT

      GlyThrAlaLeuGlyAsnIleIleSerGlyAsnThrGlyAsnValAspLeuGlnThrThr
2281  GGAACAGCATTAGGTAATATCATTTCAGGTAATACTGGAAATGTAGATTTACAAACTACT
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```
      IleSerAlaGlyIleGlyTyrThrSerGluProPheGlyIleGlyValGlyTyrAsnTyr
2341  ATTTCTGCTGGTATAGGTTATACTTCTGAGCCTTTCGGTATTGGCGTAGGTTATAACTAC

      ThrTyrValAsnProArgLeuGlyValHisThrProValLeuMetIleAsnAlaLeuAsn
2401  ACTTATGTAAATCCTAGATTAGGCGTTCATACTCCTGTACTTATGATCAATGCTTTAAAC

      AsnAsnLeuArgIleAlaValProValGlnIleAlaValSerHisAspProPheAsnAsp
2461  AACAACTTAAGAATAGCAGTTCCTGTTCAAATAGCTGTAAGTCATGATCCTTTCAATGAT

      SerAlaLysPheProTyrSerSerSerThrLysAspTyrMetGlyIleSerThrAspIle
2521  TCTGCTAAATTCCCTTATTCATCATCTACAAAAGATTATATGGGTATAAGCACTGATATA

      GlnLeuArgTyrTyrThrGlyIleAspAlaPheAsnAlaIleArgLeuTyrPheLysTyr
2581  CAATTAAGATACTATACTGGTATAGATGCTTTCAATGCTATAAGATTATACTTCAAATAC

      GlyGlnAlaGlyPheLysThrAlaAsnGlyAlaGlyAlaSerGluTyrPheAlaGlnSer
2641  GGACAAGCTGGATTTAAAACAGCTAACGGAGCTGGAGCTAGTGAGTATTTTGCTCAGTCA

      LeuGlyPheGluAlaArgPheTyrPheLeuAsnThrProValGlyAsnValThrIleAsn
2701  TTAGGTTTTGAAGCTAGATTCTATTTCTTGAATACTCCTGTTGGAAACGTAACTATCAAT

      ProPheIleLysValValTyrAsnThrAlaLeuLysGlyValSerArgThrValArgAla
2761  CCTTTCATCAAAGTTGTTTATAACACAGCTTTAAAAGGTGTAAGCAGAACTGTAAGAGCT

      GlyGluAlaValGlnAsnThrValSerGlyTyrHisProSerAsnProAsnTyrLysLeu
2821  GGAGAAGCTGTACAAAATACTGTTTCTGGTTATCATCCTTCTAATCCTAATTATAAATTA

      AspAlaPheAlaGlyArgTyrIleGlyLysAspPheLysTrpAspSerAsnProTyrAsp
2881  GATGCATTTGCTGGTAGATACATTGGTAAAGATTTCAAATGGGATTCAAATCCTTATGAT

      ValLysAlaGlnAlaValLeuGlyIleThrAlaAsnSerAspValValSerLeuTyrVal
2941  GTAAAAGCTCAGGCTGTATTAGGTATCACTGCTAACAGCGATGTAGTATCTCTTTATGTT

      GluProSerLeuGlyTyrGlnAlaThrTyrLeuGlyLysAsnIleSerGluAsnProTyr
3001  GAGCCTTCTTTAGGTTATCAAGCTACATATTTAGGAAAAAACATATCTGAAAATCCATAT

      LeuAsnIleAspSerLysValGlnHisSerLeuAlaTrpGlyAlaTyrAlaGluLeuTyr
3061  TTAAATATAGATTCTAAAGTACAACATAGCTTAGCTTGGGGTGCTTATGCAGAACTTTAT

      ValArgProValGlnAspLeuGluTrpTyrPheGluMetAspValAsnAsnGlyGlyThr
3121  GTAAGACCTGTTCAAGATCTTGAATGGTACTTCGAGATGGATGTTAATAATGGCGGTACA

      ArgGlnGluSerGlyIleProValTyrPheLysSerThrThrGlyIleThrTrpTyrLeu
3181  AGACAAGAATCTGGTATCCCTGTATACTTTAAATCTACTACAGGTATAACTTGGTATTTA

      ProAlaPheAsn
3241  CCTGCTTTCAATTAATTAGAAGTTAATTAATAGAAATTAATGAGGCTGGCCTTTAATAGG

3301  TTGGCCTCTTTTTTATTAATTTTCATATTGCAAAATAGTTTATTCATTATTATATAAGTT

3361  TACATTTTATTGTTTTGCAATATAATATTTTTATCTATAATCTACCTATATAAATTATAT
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                                                    * Presumed translational
                                                   MetLysLysIleMetLeuAla
3421  AATAGAAGAAAATATTTTATGTTTAGGAGAACAGATAAAATGAAAAAAATTATGCTGGCA

      start of Copy 7
      AlaIleAlaIleLeuThrIlePheSerAlaSerAlaLeuGlyMetTyrGlyAspGlnAsp
3481  GCTATTGCTATATTAACTATATTTAGTGCATGTGCTTTAGGAATGTATGGAGATCAAGAT

      AspTrpIleAspPheLeuThrAspGlyAsnGlnLeuArgAlaArgMetAspGlnLeuGly
3541  GACTGGATTGATTTTCTTACAGATGGAAATCAATTAAGAGCCAGAATGGATCAATTAGGA

      PheValLeuGlyAsnAsnThrIleLysGlyThrPheGlyLeuArgThrGlnAspAlaVal
3601  TTTGTACTTGGAAACAATACTATTAAAGGTACTTTCGGACTTAGAACTCAAGATGCCGTA

      ThrSerLeuGlySerIleIleSerGlyLysThrAspAsnLeuGlyLeuAspAlaThrVal
3661  ACATCATTGGGAAGTATAATTTCAGGTAAAACAGATAATTTAGGATTAGATGCTACTGTT

      SerMetGlyIleGlyTyrThrSerAspIlePheGlyIleGlyLeuGlyTyrAsnPheThr
3721  TCTATGGGAATAGGATACACTTCTGATATTTTCGGCATTGGCTTAGGATATAATTTTACA

      TyrTyrAsnSerThrLeuGlyValHisThrProValLeuMetValAsnAlaLeuAsnAsn
3781  TATTATAACAGCACTTTAGGCGTTCATACTCCTGTACTTATGGTCAATGCTTTAAATAAT

      AsnLeuArgIleAlaIleProIleGlnIleAlaAlaSerLysAspProPheGlyLysTyr
3841  AATTTAAGAATAGCAATACCTATACAAATAGCTGCATCAAAAGATCCTTTCGGAAAATAT

      ThrIleSerGlnTyrLysAspTyrLeuGlyIleSerThrAspIleGlnIleArgTyrTyr
3901  ACTATCAGTCAATATAAAGACTATTTAGGAATAAGCACAGATATACAAATAAGATACTAT

      ThrGluIleAspValPheAsnGlnValArgLeuTyrIleLysTyrGlyGlnSerGlyTyr
3961  ACAGAAATAGATGTATTCAATCAAGTAAGATTATACATCAAATATGGACAGTCAGGTTAT

      LysAsnValLysAsnAsnPheAspMetPheAlaGlnSerPheGlyPheGluThrArgLeu
4021  AAAAATGTTAAAAATAATTTTGATATGTTTGCTCAATCATTTGGTTTTGAAACTAGACTA

      TyrPheLeuAsnArgThrIleGlyAsnValAsnIleAsnProPheIleLysValSerTyr
4081  TATTTCTTAAACCGCACAATTGGAAATGTAAATATTAATCCTTTTATTAAAGTTTCATAT

      AsnThrAlaLeuAlaSerSerAspValMetValArgAlaGlyGluSerLeuValAsnThr
4141  AATACAGCTTTAGCCAGCAGTGATGTAATGGTTAGAGCAGGAGAATCTCTTGTAAATACT

      ThrTyrSerLysLysGluAsnLysTrpGluLysAsnProTyrAsnValThrAlaAlaAla
4201  ACTTATAGTAAAAAAGAAAATAAATGGGAAAAAAATCCTTATAATGTAACTGCTGCTGCT

      ValLeuGlyLeuThrAlaAsnSerAspMetLeuSerLeuCysValGluProSerLeuGly
4261  GTATTAGGATTAACTGCTAACAGTGATATGCTATCTCTTTGTGTTGAACCTTCTTTAGGA

      TyrAsnAlaValTyrLysGlyLysTyrLysThrAspSerLysTyrTyrLysValGlnHis
4321  TACAATGCCGTTTATAAAGGAAAATATAAAACTGATAGTAAATATTATAAAGTACAGCAT

      AsnLeuTyrTrpGlyAlaTyrAlaGluLeuTyrIleThrProValGlnAspIleGluTrp
4381  AATTTATATTGGGGAGCTTATGCAGAACTTTATATTACTCCCGTTCAAGATATTGAATGG
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      TyrPheGluMetAspIleAsnAsnGlyAsnSerArgGlnThrSerSerIleProIleTyr
4441  TATTTTGAAATGGACATTAATAATGGTAATTCAAGACAGACTTCTTCTATACCTATATAC

      PheGluSerThrThrGlyIleThrTrpTyrLeuProGluLeu
4501  TTTGAATCTACTACAGGGATAACTTGGTATTTGCCTGAATTATAATAAAAATATTATTTT

4561  GTATAATTTAAACATTTATTGAATAAAAAAAATAAAAAATTAATAATTCTATAAAAAATT

4621  TAAATTAATACTTTACATATTTAAATAAATAGCTGTTTTTATTAAATCTAAGTATTATTT

4681  TAAAAAATAATAATTTTATTTATATTAGTGTTGACTTTTTTATGTAAATTCATTAGAATA

4741  AAAGTGTTGGTAAGATATAGACATTAAAAAAAGATTGCTTATAATGTCTATAACCATATT

4801  AGGAACAATAAAGATCCTCTAAATCCTTATTATTCCAATTGACACATTAGTAAAAACAGC

4861  ATAATTTTTCCATTTAAATATCTGTTTTTACGGAGAATAAAGATCCTCTAAATCTTATTA

4921  TTCTCACTGATTTTTTATAATCCGTTAGAATTATATAGATGTTCTAACTCATCTGGAAAT

4981  AACAAAGATCCTCTGTCTTTGTTGTTTCCTAAAATTATTTTGGAGTTACTACTTACAATG

                                          *Presumed translational start
                                        MetLysLysValLeuLeuThrAlaMet
5041  AGTATTAACTATATAAAATTTTAGGAGAAAAATAATGAAAAAAGTTTTATTGACAGCTAT

      of Copy 5                                  * Amino terminus of Copy 5
      AlaLeuLeuThrIleAlaSerAlaSerAlaPheGlyMetTyrGlyAspArgAspSerTrp
5101  GGCATTATTGACTATAGCTAGTGCATCTGCTTTCGGTATGTATGGCGACAGAGATTCTTG

      IleAspPheLeuThrHisGlyAsnGlnPheArgAlaArgMetAspGlnLeuGlyPheVal
5161  GATCGACTTCCTTACTCATGGTAATCAGTTCAGAGCTAGAATGGATCAATTAGGTTTCGT

      LeuGlyAsnGlyThrIleLysGlyThrPheGlyPheArgSerGlnAlaIleGlyThrAla
5221  TTTAGGTAACGGTACTATTAAAGGTACTTTCGGTTTTAGATCTCAAGCTATTGGAACAGC

      LeuGlyAsnIleIleSerGlyAsnThrGlyAsnValAspLeuGlnThrThrIleSerAla
5281  ATTAGGTAATATCATTTCAGGTAATACTGGAAATGTAGATTTACAAACTACTATTTCTGC

      GlyIleGlyTyrThrSerGluProPheGlyIleGlyValGlyTyrAsnTyrThrTyrVal
5341  TGGTATAGGTTATACTTCTGAGCCTTTCGGTATTGGCGTAGGTTATAACTACACTTATGT

      AsnProArgLeuGlyValHisThrProValLeuMetIleAsnAlaLeuAsnAsnAsnLeu
5401  AAATCCTAGATTAGGCGTTCATACTCCTGTACTTATGATCAATGCTTTAAACAACAACTT

      ArgIleAlaValProValGlnIleAlaValSerHisAspProPheAsnAspSerAlaLys
5461  AAGAATAGCAGTTCCTGTTCAAATAGCTGTAAGTCATGATCCTTTCAATGATTCTGCTAA

      PheProTyrSerSerSerThrLysAspTyrMetGlyIleSerThrAspIleGlnLeuArg
5521  ATTCCCTTATTCATCATCTACAAAAGATTATATGGGTATAAGCACTGATATACAATTAAG

      TyrTyrThrGlyIleAspAlaPheAsnAlaIleArgLeuTyrPheLysTyrGlyGlnAla
5581  ATACTATACTGGTATAGATGCTTTCAATGCTATAAGATTATACTTCAAATACGGACAAGC
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GlyPheLysThrAlaAsnGlyAlaSerGluTyrPheAlaGlnSerLeuGlyPheGluAla
5641 TGGATTTAAAACAGCTAACGGAGCTAGTGAGTATTTTGCTCAGTCATTAGGTTTTGAAGC

ArgPheTyrPheLeuAsnThrProValGlyAsnValThrIleAsnProPheIleLysVal
5701 TAGATTCTATTTCTTGAATACTCCTGTTGGAAACGTAACTATCAATCCTTTCATCAAAGT

ValTyrAsnThrAlaLeuLysGlyValSerArgThrValArgAlaGlyGluAlaValGln
5761 TGTTTATAACACAGCTTTAAAAGGTGTAAGCAGAACTGTAAGAGCTGGAGAAGCTGTACA

AsnThrValSerGlyTyrAsnProTyrAspProAsnTyrLysLeuAspAlaPheAlaGly
5821 AAATACTGTTTCTGGTTATAATCCTTATGATCCTAATTATAAATTAGATGCATTTGCTGG

ArgTyrIleGlyLysAspPheLysTrpAspSerAsnProTyrAspValLysAlaGlnAla
5881 TAGATACATTGGTAAAGATTTCAAATGGGATTCAAATCCTTATGATGTAAAAGCTCAGGC

ValLeuGlyIleThrAlaAsnSerAspValValSerLeuTyrValGluProSerLeuGly
5941 TGTATTAGGTATCACTGCTAACAGCGATGTAGTATCTCTTTATGTTGAGCCTTCTTTAGG

TyrGlnAlaThrTyrLeuGlyLysHisIleSerGluAsnProTyrLeuAsnIleAspSer
6001 TTATCAAGCTACATATTTAGGAAAACACATATCTGAAAATCCATATTTAAATATAGATTC

LysValGlnHisSerLeuAlaTrpGlyAlaTyrAlaGluLeuTyrValArgProValGln
6061 TAAAGTACAACATAGCTTAGCTTGGGGTGCTTATGCAGAACTTTATGTAAGACCTGTTCA

AspLeuGluTrpTyrPheGluMetAspIleAsnAsnSerAspSerLysArgAsnGlyVal
6121 AGATCTTGAATGGTACTTCGAGATGGACATCAATAACTCTGATTCAAAAAGAAATGGTGT

ProValAsnPheAlaThrSerThrGlyIleThrTrpTyrLeuProAlaLeuGlyGlyAla
6181 TCCTGTTAACTTCGCAACTTCTACAGGTATAACTTGGTACTTACCTGCTTTAGGCGGTGC

Gln
6241 TCAATAATTAATTTCTGTTAATTAAAGAATTTACAGAGGCTGGCTTTAATAAAAAAGTCA

6301 GTCTCTTTTTTTATCGCATATTTTCATATAATTAAACAAAAATATTTACATTATATAATT

Appendix 3

Nucleotide sequence of T. hyo gene insert of pTrep106

```
(linker) <--T. hyo DNA --
   1  GAATTCC CTATTTTATATTTTTAGTATAATAAAAAATATAAACTTCAATTTAGGTATGTATA
      CTTAAGG GATAAAATATAAAAATCATATTATTTTTTATATTTGAAGTTAAATCCATACATAT

      1 ECOR1

        (Signal peptide sequence -->
        MetLysLysPhePheLeuIleMetThrValLeuLeuSerMetSerTyrCysSerIlePhe
  63    ATGAAAAAGTTTTTTCTAATTATGACAGTATTATTAAGTATGTCATATTGTTCAATTTTT
        TACTTTTTCAAAAAAGATTAATACTGTCATAATAATTCATACAGTATAACAAGTTAAAAA

           <--begin mature protein -->
        GlyMetTyrGlyAspGlnAspAspTrpIleAspPheLeuThrAspGlyAsnGlnPheArg
 123    GGTATGTATGGAGATCAGGACGATTGGATTGATTTTCTTACAGACGGCAATCAGTTTAGA
        CCATACATACCTCTAGTCCTGCTAACCTAACTAAAAGAATGTCTGCCGTTAGTCAAATCT

        AlaArgMetAspGlnLeuGlyPheValLeuGlyAsnSerThrIleLysGlyThrPheGly
 183    GCTAGAATGGATCAATTAGGATTTGTTTTAGGTAATAGTACTATTAAAGGTACTTTCGGT
        CGATCTTACCTAGTTAATCCTAAACAAAATCCATTATCATGATAATTTCCATGAAAGCCA

        PheArgThrGlnSerSerSerThrGlnLeuGlyTyrIleLeuLeuAsnAsnAsnLeuGly
 243    TTTAGAACTCAAAGTTCATCAACTCAATTAGGATATATTTTGTTGAATAATAATCTTGGT
        AAATCTTGAGTTTCAAGTAGTTGAGTTAATCCTATATAAAACAACTTATTATTAGAACCA

        ThrTyrLeuGlyAlaThrIleSerGlyGlyIleGlyTyrThrSerGluAlaPheSerIle
 303    ACTTATTTGGGAGCAACTATTTCTGGCGGTATAGGATATACTTCTGAGGCTTTTAGTATA
        TGAATAAACCCTCGTTGATAAAGACCGCCATATCCTATATGAAGACTCCGAAAATCATAT

        GlyIleGlyTyrAsnTyrThrSerHisSerLeuProThrSerAspAsnPheGlySer
 363    GGCATAGGCTATAATTATACCAGCCATTCCTTATTTCCTACTAGCGATAACTTTGGTTCT
        CCGTATCCGATATTAATATGGTCGGTAAGGAATAAAGGATGATCGCTATTGAAACCAAGA

        HisThrProValLeuMetIleAsnAlaLeuAsnAspAsnLeuArgIleValIleProVal
 423    CATACTCCAGTACTTATGATTAATGCTTTAAATGATAATTTGAGGATAGTTATTCCTGTG
        GTATGAGGTCATGAATACTAATTACGAAATTTACTATTAAACTCCTATCAATAAGGACAC

        GlnIleLeuValHisAsnGluSerIleAspGlnLeuGlyTyrTyrArgAspAsnTyrLeu
 483    CAAATATTAGTACATAATGAAAGTATTGATCAACTTGGTTACTATAGAGATAATTATTTA
        GTTTATAATCATGTATTACTTTCATAACTAGTTGAACCAATGATATCTCTATTAATAAAT

        GlyIleSerThrAspThrGlnIleArgTyrTyrThrGlyIleAspAlaPheAsnGluIle
 543    GGTATAAGTACTGATACGCAAATAAGATATTATACAGGCATAGATGCTTTTAATGAAATA
        CCATATTCATGACTATGCGTTTATTCTATAATATGTCCGTATCTACGAAAATTACTTTAT

        ArgLeuTyrValLysTyrGlyGlnLeuGlyTyrLysIleAsnProHisAspThrIleAsn
 603    AGATTATATGTAAAATATGGGCAATTAGGATATAAAATTAATCCGCATGATACTATAAAT
        TCTAATATACATTTTATACCCGTTAATCCTATATTTTAATTAGGCGTACTATGATATTTA

        TyrThrGlnGluValLeuAlaArgSerPheGlyPheGluThrArgPheTyrPheLeuAsn
 663    TATACACAAGAAGTTTTAGCAAGATCATTTGGTTTTGAAACAAGATTCTATTTTTTGAAT
        ATATGTGTTCTTCAAAATCGTTCTAGTAAACCAAAACTTTGTTCTAAGATAAAAAACTTA
```

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                ThrAlaValGlyAsnValThrIleAsnProPheIleLysValAlaTyrAsnThrAlaLeu
723             ACTGCTGTTGGAAATGTAACTATCAATCCTTTTATTAAAGTAGCATATAATACAGCTTTG
                TGACGACAACCTTTACATTGATAGTTAGGAAAATAATTTCATCGTATATTATGTCGAAAC

                HisGlyTyrSerThrMetValArgAlaLeuAspGlyMetTyrGluGluIleGluGlyTyr
783             CATGGATATAGTACCATGGTAAGAGCATTGGATGGTATGTATGAAGAAATAGAAGGTTAT
                GTACCTATATCATGGTACCATTCTCGTAACCTACCATACATACTTCTTTATCTTCCAATA

                TyrProAspSerProAlaGlnSerTyrGluAspIleAsnValLysTrpAspLysAsnPro
843             TATCCAGATAGTCCTGCTCAATCATATGAAGATATTAATGTTAAATGGGATAAGAATCCT
                ATAGGTCTATCAGGACGAGTTAGTATACTTCTATAATTACAATTTACCCTATTCTTAGGA

                TyrAspValThrValGlnAlaValLeuGlyValThrAlaAsnSerAspIleValSerLeu
903             TATGATGTAACTGTGCAGGCAGTATTGGGAGTAACTGCTAATAGCGATATAGTATCACTT
                ATACTACATTGACACGTCCGTCATAACCCTCATTGACGATTATCGCTATATCATAGTGAA

                TyrValGluProSerLeuGlyTyrArgAlaLysTyrLeuGlyLysLeuThrTyrGluAsp
963             TATGTTGAGCCTTCTTTAGGTTATAGGGCTAAATATTTAGGAAAATTAACATATGAAGAT
                ATACAACTCGGAAGAAATCCAATATCCCGATTTATAAATCCTTTTAATTGTATACTTCTA

                ProAspGlyLysValAsnPheAspPheLysValAsnHisTyrLeuSerTrpGlyAlaTyr
1023            CCAGATGGAAAAGTTAATTTTGATTTTAAAGTTAATCATTATTTATCTTGGGGTGCTTAT
                GGTCTACCTTTTCAATTAAAACTAAAATTTCAATTAGTAATAAATAGAACCCCACGAATA

                AlaGluLeuTyrIleThrProValLysAspLeuGluTrpTyrPheGluMetAspValAsn
1083            GCAGAGCTTTATATAACACCGGTAAAAGATTTAGAATGGTATTTTGAAATGGATGTTAAT
                CGTCTCGAAATATATTGTGGCCATTTTCTAAATCTTACCATAAAACTTTACCTACAATTA

                AsnSerAspSerAspSerThrGlyIleProValSerPheAlaSerThrThrGlyIleThr
1143            AATAGTGATTCAGATTCTACAGGTATACCTGTTAGTTTTGCTTCTACTACAGGAATAACT
                TTATCACTAAGTCTAAGATGTCCATATGGACAATCAAAACGAAGATGATGTCCTTATTGA

                TrpTyrLeuProGluPheOC
1203            TGGTATTTGCCAGAATTTTAATTATAAAGCAAATTTTATATGATAAAATAAAAAATGTGG
                ACCATAAACGGTCTTAAAATTAATATTTCGTTTAAAATATACTATTTTATTTTTTACACC

1263            GGTATTTATTATTAAAAAATAAATACCCCACATTTTATTAAATAATTTTTTCAGTAATTT
                CCATAAATAATAATTTTTTATTTATGGGGTGTAAAATAATTTATTAAAAAAGTCATTAAA

                                        (begin gene encoding a related protein-->
                                                                        Met
1323            TACATTTATATATTTTTTAGTATAATAAAAATATAAACTTAAATTTAGGTATATACAATG
                ATGTAAATATATAAAAAATCATATTATTTTTATATTTGAATTTAAATCCATATATGTTAC

                LysLysIlePheLeuIleMetThrValLeuLeuSerMetSerTyrCysSerIlePheGly
1383            AAAAAAATTTTTCTAATTATGACAGTATTATTAAGTATGTCATATTGTTCAATATTTGGT
                TTTTTTTAAAAAGATTAATACTGTCATAATAATTCATACAGTATAACAAGTTATAAACCA

                                                                        (linker)
                MetTyrGlyAspGlnAspAspTrpIleAspPheLeuIleAspGlyAsnGlnPheArg
1443            ATGTATGGAGATCAGGACGATTGGATTGATTTTCTTATAGACGGTAATCAGTTTAGAG GGAATTC
                TACATACCTCTAGTCCTGCTAACCTAACTAAAAGAATATCTGCCATTAGTCAAATCTC CCTTAAG

                                                                1502 ECOR1
```

Appendix 4

Partial DNA sequence of expression plasmid pTrep301

```
                (plasmid pUC9-->      <-- E. coli lacZ ------------ | -T. hyo
                                      MetThrMetIleThrProSerLeuArgAlaSerTyrCys
    1   AATTTCACACAGGAAACAGCTATGACCATGATTACGCCAAGCTTGCATGCCTCATATTGT
        TTAAAGTGTGTCCTTTGTCGATACTGGTACTAATGCGGTTCGAACGTACGGAGTATAACA
                                                          39 HINDIII.

        signal ---->|<--mature T. hyo 39 kDa protein-->
        SerIlePheGlyMetTyrGlyAspGlnAspAspTrpIleAspPheLeuThrAspGlyAsn
   61   TCAATTTTTGGTATGTATGGAGATCAGGACGATTGGATTGATTTTCTTACAGACGGCAAT
        AGTTAAAAACCATACATACCTCTAGTCCTGCTAACCTAACTAAAAGAATGTCTGCCGTTA

        GlnPheArgAlaArgMetAspGlnLeuGlyPheValLeuGlyAsnSerThrIleLysGly...
  121   CAGTTTAGAGCTAGAATGGATCAATTAGGATTTGTTTTAGGTAATAGTACTATTAAAGGT...
        GTCAAATCTCGATCTTACCTAGTTAATCCTAAACAAAATCCATTATCATGATAATTTCCA...
```

The first 25 amino acids of this recombinant fusion protein were determined on protein isolated from E. coli transformed with plasmid pTrep301. The protein was isolated as a Triton X-100 insoluble aggregate and was further purified by preparative SDS gel electrophoresis.

Appendix.

--Predicted amino acid sequence from PCR derived T. hyo (B204) clones

pTrep605--Copy2

```
        10         20         30         40         50         60
MTMITNRGSM YGDQDDWIDF LTDGNQFRAR MDQLGFVLGN STIKGTFGFR SQSLSTQLGY

        70         80         90        100        110        120
ILAIYKDYTY LGATISGGIG YTSEAFSIGL GYNYTTPLPI SYNFGSHTPV LMINALNDNL

       130        140        150        160        170        180
RIVIPVQILV HDGNMNMTDN INYLYNFLGI STDTQIRYYT GIDAFNEIRL YVKYGQLGYK

       190        200        210        220        230        240
GGSYTDKSYD EEFFARSFGF ETRFYFLNTA VGNVTINPFI KVAYNTALHG FSTMVRSLDS

       250        260        270        280        290        300
VIEEIEGYSS DRTAKAAGNI NAKWDKNPYD VTVQAVLGVT ANSDIVSLYV EPSLGYRAKY

       310        320        330        340        350        360
LGKLTYEDPD GKVNFDFKVN HYLSWCAYAE LYITPVKDLE WYFEMDVNNS DSDSTGIPVS

       370
FASTTGITWY LPEF
```

pTrep604--Copy3

```
        10         20         30         40         50         60
MTMITNRGSM YGDQDDWIDF LTDGNQFRAR MDQFGFVLGN STIKGTFGFR SQSLSTQLGY

        70         80         90        100        110        120
ILAIYKDYTY LGATISGGIG YTSEAFSIGL GYNYTTPLPI SDNFGSHTPV LMINALNDNL

       130        140        150        160        170        180
RIVIPVQILV YNGNVQKVDK QGNISYSHDY LGISTDTQIR YYTGIDAFNE IRLYVKYGQL

       190        200        210        220        230        240
GYKNAPYVGK NYEEEFFSRS FGFETRFYFL NTAVGNVTIN PFIKVAYNTA LHGFSTMIRA

       250        260        270        280        290        300
LDSMFEPIEG YSSDRPVSSQ ANINAKWDKN PYDVTVQAVL GVTANSDIVS LYVEPSLGYR

       310        320        330        340        350        360
AKYLGKLTYE DPDGKVNLDF KVNHYLSWGA YAELYITPVK DLEWYFEMDV NNSDSDSTGI

       370     377
PVSFASTTGI TWYLPEF
```

pTrep345--Copy4

```
        10          20          30          40          50          60
MTMITNRGSM YGDQDDWIDF LTDGNQFRAR MDQFGFVLGN NTIKGTFGFR SQSLSTHLGY

        70          80          90         100         110         120
ILLNNNFGTY FGTTISCGIG YTSEAFSIGI GYNYTTPLPI SDNFGSHTPV LMINALNDNL

       130         140         150         160         170         180
RIVIPVQILV YNGNIQKVDK QGNIHDTYDY LGISTDTQIR YYTGIDAFNE IRLYIKYGQL

       190         200         210         220         230         240
GYKNAPYVGK NYEEELFSRS FGFETRFYFL NTTVGNVTIN PFIKVAYNTA LHGVGTMIRA

       250         260         270         280         290         300
LDTMLQPIED YYPDRPVSSQ VDIDYKLDKN PYDVTVQAVL GVTANSDIVS LYVEPSLGYK

       310         320         330         340         350         360
AKYLGKMQDE KVNLDFKVNH YLSWGAYAEL YITPVKDLEW YFEMDVNNSD SDSTGIPVSF

       370
ASTTGITWYL PEF
```

pTrep613--Copy5

```
        10          20          30          40          50          60
MTMITNRGSM YGDRDSWIDF LTHGNQFRAR MDQLGFVLGN GTIKGTFGFR SQAIGTALGN

        70          80          90         100         110         120
IISGNTGNVD LQTTISAGIG YTSEPFGIGV GYNYTYVNPR LGVHTPVLMI NALNNNLRIA

       130         140         150         160         170         180
VPVQIAVSHD PFNDSAKFPY SSSTKDYMGI STDIQLRYYT GIDAFNAIRL YFKYGQAGFK

       190         200         210         220         230         240
TANGASEYFA QSLGFEARFY FLNTPVGNVT INPFIKVVYN TALKGVSRTV RAGEAVQNTV

       250         260         270         280         290         300
SGYNPYDPNY KLDAFAGRYI GKDFKWDSNP YDVKAQAVLG ITANSDVVSL YVEPSLGYQA

       310         320         330         340         350         360
TYLGKHISEN PYLNIDSKVQ HSLAWGAYAE LYVRPVQDLE WYFEMDINNS DSKRNGVPVN

       370       378
FATSTGITWY LPALGGAQ
```

pTrep620--Copy7

```
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        70         80         90        100        110        120
IISGKTDNLG LDATVSMGIG YTSDIFGIGL GYNFTYYNST LGVHTPVLMV NALNNNLRIA

       130        140        150        160        170        180
IPIQIAASKD PFGKYTISQY KDYLGISTDI QIRYYTEIDV FNQVRLYIKY GQSGYKNVKN

       190        200        210        220        230        240
NFDMFAQSFG FETRLYFLNR TIGNVNINPF IKVSYNTALA SSDVMVRAGE SLVNTTYSKK

       250        260        270        280        290        300
ENKWEKNPYN VTAAAVLGLT ANSDMLSLCV EPSLGYNAVY KGKYKTDSKY YKVQHNLYWG

       310        320        330        340        349
AYAELYITPV QDIEWYFEMD INNGNSRQTS SIPIYFESTT GITWYLPEF
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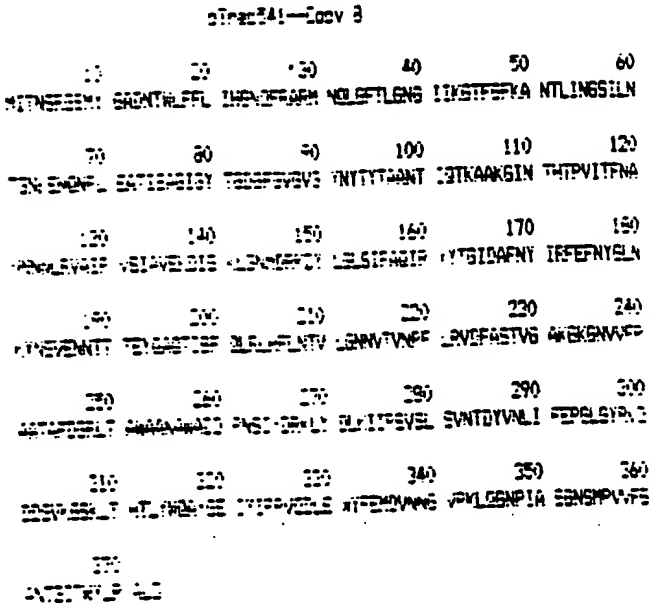

Appendix #6--Predicted amino acid sequence of pTrep702 recombinant product

```
                                          <------Protein derived frc
      MetThrMetIleThrProSerLeuHisAlaCysArgSerThrLeuGluAspProArgVa
    1 ATGACCATGATTACGCCAAGCTTGCATGCCTGCAGGTCGACTCTAGAGGATCCCCGGGT

      pUC polylinker------>||<---Copy 6 ORF--->
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   61 CCGAGCTCGAATTGGGAATTCCCTATGGCATTATTGACTATAGCTAGTGCATCTGCTTT

      GlyMetTyrGlyAspArgAspSerTrpIleAspPheLeuThrHisGlyAsnGlnPheAr
  121 GGTATGTACGGCGACAGAGATTCTTGGATCGACTTCCTTACTCATGGTAATCAGTTCAG

      AlaArgMetAspGlnLeuGlyPheValLeuGlyAsnGlyThrIleLysGlyThrPheGl
  181 GCTAGAATGGATCAATTAGGTTTCGTTTTAGGTAACGGTACTATTAAAGGTACTTTCGG

      PheArgSerGlnAlaIleGlyThrAlaLeuGlyAsnIleIleSerGlyAsnThrGlyAs
  241 TTTAGATCTCAAGCTATTGGAACAGCATTAGGTAATATCATTTCAGGTAATACTGGAAA

      ValAspLeuGlnThrThrIleSerAlaGlyIleGlyTyrThrSerGluProPheGlyIl
  301 GTAGATTTACAAACTACTATTTCTGCTGGTATAGGTTATACTTCTGAGCCTTTCGGTAT

      GlyValGlyTyrAsnTyrThrTyrValAsnProArgLeuGlyValHisThrProValLe
  361 GGCGTAGGTTATAACTACACTTATGTAAATCCTAGATTAGGCGTTCATACTCCTGTACT

      MetIleAsnAlaLeuAsnAsnAsnLeuArgIleAlaValProValGlnIleAlaValSe
  421 ATGATCAATGCTTTAAACAACAACTTAAGAATAGCAGTTCCTGTTCAAATAGCTGTAAG

      HisAspProPheAsnAspSerAlaLysPheProTyrSerSerSerThrLysAspTyrMe
  481 CATGATCCTTTCAATGATTCTGCTAAATTCCCTTATTCATCATCTACAAAAGATTATAT

      GlyIleSerThrAspIleGlnLeuArgTyrTyrThrGlyIleAspAlaPheAsnAlaIl
  541 GGTATAAGCACTGATATACAATTAAGATACTATACTGGTATAGATGCTTTCAATGCTAT

      ArgLeuTyrPheLysTyrGlyGlnAlaGlyPheLysThrAlaAsnGlyAlaGlyAlaSe
  601 AGATTATACTTCAAATACGGACAAGCTGGATTTAAAACAGCTAACGGAGCTGGAGCTAG

      GluTyrPheAlaGlnSerLeuGlyPheGluAlaArgPheTyrPheLeuAsnThrProVa
  661 GAGTATTTTGCTCAGTCATTAGGTTTTGAAGCTAGATTCTATTTCTTGAATACTCCTGT

      GlyAsnValThrIleAsnProPheIleLysValValTyrAsnThrAlaLeuLysGlyVa
  721 GGAAACGTAACTATCAATCCTTTCATCAAAGTTGTTTATAACACAGCTTTAAAAGGTGT

      SerArgThrValArgAlaGlyGluAlaValGlnAsnThrValSerGlyTyrHisProSe
  781 AGCAGAACTGTAAGAGCTGGAGAAGCTGTACAAAATACTGTTTCTGGTTATCATCCTTC

      AsnProAsnTyrLysLeuAspAlaPheAlaGlyArgTyrIleGlyLysAspPheLysTr
  841 AATCCTAATTATAAATTAGATGCATTTGCTGGTAGATACATTGGTAAAGATTTCAAATG

      AspSerAsnProTyrAspValLysAlaGlnAlaValLeuGlyIleThrAlaAsnSerAs
  901 GATTCAAATCCTTATGATGTAAAAGCTCAGGCTGTATTAGGTATCACTGCTAACAGCGA

      ValValSerLeuTyrValGluProSerLeuGlyTyrGlnAlaThrTyrLeuGlyLysAs
  961 GTAGTATCTCTTTATGTTGAGCCTTCTTTAGGTTATCAAGCTACATATTTAGGAAAAAA
```

```
                                    ---End of Copy 6 ORF --->||<---beta
     IleSerGluAsnProTyrLeuAsnIleAspSerLysValGlnHisArgGluPheGlyAsn
1021 ATATCTGAAAATCCATATTTAAATATAGATTCTAAAGTACAACATAGGGAATTCGGCAAT

     galactosidase--->
     SerLeuAlaValValLeuGlnArgArgAspTrpGluAsnProGlyValThrGlnLeuAsn
1081 TCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAAT

     ArgLeuAlaAlaHisProProPheAlaSerTrpArgAsnSerGluGluAlaArgThrAsp
1141 CGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGAT

     ArgProSerGlnGlnLeuArgSerLeuAsnGlyGluTrpArgLeuMetArgTyrPheLeu
1201 CGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGCGCCTGATGCGGTATTTTCTC

     LeuThrHisLeuCysGlyIleSerHisArgIleTrpCysThrLeuSerThrIleCysSer
1261 CTTACGCATCTGTGCGGTATTTCACACCGCATATGGTGCACTCTCAGTACAATCTGCTCT

     AspAlaAlaAM
1321 GATGCCGCATAGTTAAGCCAGCCCCGACACCCGCCAACACCCGCTGACGCGCCCTGACGG

1381 GCTTGTCTGCTCCCGGCATCCGCTTACAGACAAGCTGTGACCGTCTCCGGGAGCTGCATG
```

Appendix #7--Predicted protein sequence of pTrep704 recombinant product

```
                                          <----------Protein derived from
    MetThrMetIleThrProSerLeuHisAlaCysArgSerThrLeuGluAspProArgVal
  1 ATGACCATGATTACGCCAAGCTTGCATGCCTGCAGGTCGACTCTAGAGGATCCCCGGGTA

    pUC polylinker------>||<------Copy 6 ORF
    ProSerSerAsnTrpGluPheProMetAlaLeuLeuThrIleAlaSerAlaSerAlaPhe
 61 CCGAGCTCGAATTGGGAATTCCCTATGGCATTATTGACTATAGCTAGTGCATCTGCTTTC

    GlyMetTyrGlyAspArgAspSerTrpIleAspPheLeuThrHisGlyAsnGlnPheArg
121 GGTATGTACGGCGACAGAGATTCTTGGATCGACTTCCTTACTCATGGTAATCAGTTCAGA

    AlaArgMetAspGlnLeuGlyPheValLeuGlyAsnGlyThrIleLysGlyThrPheGly
181 GCTAGAATGGATCAATTAGGTTTCGTTTTAGGTAACGGTACTATTAAAGGTACTTTCGGT

    PheArgSerGlnAlaIleGlyThrAlaLeuGlyAsnIleIleSerGlyAsnThrGlyAsn
241 TTTAGATCTCAAGCTATTGGAACAGCATTAGGTAATATCATTTCAGGTAATACTGGAAAT

    ValAspLeuGlnThrThrIleSerAlaGlyIleGlyTyrThrSerGluProPheGlyIle
301 GTAGATTTACAAACTACTATTTCTGCTGGTATAGGTTATACTTCTGAGCCTTTCGGTATT

    GlyValGlyTyrAsnTyrThrTyrValAsnProArgLeuGlyValHisThrProValLeu
361 GGCGTAGGTTATAACTACACTTATGTAAATCCTAGATTAGGCGTTCATACTCCTGTACTT

    MetIleAsnAlaLeuAsnAsnAsnLeuArgIleAlaValProValGlnIleAlaValSer
421 ATGATCAATGCTTTAAACAACAACTTAAGAATAGCAGTTCCTGTTCAAATAGCTGTAAGT

    HisAspProPheAsnAspSerAlaLysProTyrSerSerSerThrLysAspTyrMet
481 CATGATCCTTTCAATGATTCTGCTAAATTCCCTTATTCATCATCTACAAAAGATTATATG

    GlyIleSerThrAspIleGlnLeuArgTyrTyrThrGlyIleAspAlaPheAsnAlaIle
541 GGTATAAGCACTGATATACAATTAAGATACTATACTGGTATAGATGCTTTCAATGCTATA

    ArgLeuTyrPheLysTyrGlyGlnAlaGlyPheLysThrAlaAsnGlyAlaGlyAlaSer
601 AGATTATACTTCAAATACGGACAAGCTGGATTTAAAACAGCTAACGGAGCTGGAGCTAGT

    GluTyrPheAlaGlnSerLeuGlyPheGluAlaArgPheTyrPheLeuAsnThrProVal
661 GAGTATTTTGCTCAGTCATTAGGTTTTGAAGCTAGATTCTATTTCTTGAATACTCCTGTT

    GlyAsnValThrIleAsnProPheIleLysValValTyrAsnThrAlaLeuLysGlyVal
721 GGAAACGTAACTATCAATCCTTTCATCAAAGTTGTTTATAACACAGCTTTAAAAGGTGTA

    SerArgThrValArgAlaGlyGluAlaValGlnAsnThrValSerGlyTyrHisProSer
781 AGCAGAACTGTAAGAGCTGGAGAAGCTGTACAAAATACTGTTTCTGGTTATCATCCTTCT

    AsnProAsnTyrLysLeuAspAlaPheAlaGlyArgTyrIleGlyLysAspPheLysTrp
841 AATCCTAATTATAAATTAGATGCATTTGCTGGTAGATACATTGGTAAAGATTTCAAATGG

    AspSerAsnProTyrAspValLysAlaGlnAlaValLeuGlyIleThrAlaAsnSerAsp
901 GATTCAAATCCTTATGATGTAAAAGCTCAGGCTGTATTAGGTATCACTGCTAACAGCGAT

    ValValSerLeuTyrValGluProSerLeuGlyTyrGlnAlaThrTyrLeuGlyLysAsn
961 GTAGTATCTCTTTATGTTGAGCCTTCTTTAGGTTATCAAGCTACATATTTAGGAAAAAAC
```

```
     IleSerGluAsnProTyrLeuAsnIleAspSerLysValGlnHisSerLeuAlaTrpGly
1021 ATATCTGAAAATCCATATTTAAATATAGATTCTAAAGTACAACATAGCTTAGCTTGGGGT

     AlaTyrAlaGluLeuTyrValArgProValGlnAspLeuGluTrpTyrPheGluMetAsp
1081 GCTTATGCAGAACTTTATGTAAGACCTGTTCAAGATCTTGAATGGTACTTCGAGATGGAT

     ValAsnAsnGlyGlyThrArgGlnGluSerGlyIleProValTyrPheLysSerThrThr
1141 GTTAATAATGGCGGTACAAGACAAGAATCTGGTATCCCTGTATACTTTAAATCTACTACA

     GlyIleThrTrpTyrLeuProAlaPheAsnOC
1201 GGTATAACTTGGTATTTACCTGCTTTCAATTAATTAGAAGTTAATTAATAGAAATTAATG
```

Appendix #8--Predicted protein sequence of recombinant product of pTrep505

```
                                                    <---Protein derived from
      MetThrMetIleThrProSerLeuHisAlaCysArgSerThrLeuGluAspProArgVal
    1 ATGACCATGATTACGCCAAGCTTGCATGCCTGCAGGTCGACTCTAGAGGATCCCCGGGTA

      pUC polylinker------>||<---Copy 2 ORF --->
      ProSerSerAsnTrpGluPheProArgMetAspGlnLeuGlyPheValLeuGlyAsnSer
   61 CCGAGCTCGAATTGGGAATTCCCTAGAATGGATCAATTAGGATTTGTTTTAGGTAATAGC

      ThrIleLysGlyThrPheGlyPheArgSerGlnSerLeuSerThrGlnLeuGlyTyrIle
  121 ACCATTAAAGGTACTTTCGGTTTTAGATCTCAGAGTTTATCAACTCAATTAGGATATATT

      LeuAlaIleTyrLysAspTyrThrTyrLeuGlyAlaThrIleSerGlyGlyIleGlyTyr
  181 TTGGCTATATATAAAGATTATACTTATTTAGGAGCAACTATTTCCGGCGGTATAGGATAT

      ThrSerGluAlaPheSerIleGlyLeuGlyTyrAsnTyrThrThrProLeuProIleSer
  241 ACTTCTGAGGCTTTTAGTATAGGTTTAGGTTATAATTATACTACACCGCTTCCTATTAGT

      TyrAsnPheGlySerHisThrProValLeuMetIleAsnAlaLeuAsnAspAsnLeuArg
  301 TATAACTTTGGTTCTCATACTCCTGTACTTATGATTAATGCTTTAAATGATAATTTGAGG

      IleValIleProValGlnIleLeuValHisAspGlyAsnMetAsnMetThrAspAsnIle
  361 ATAGTTATTCCTGTACAAATATTAGTACATGATGGTAATATGAATATGACGGATAATATT

      AsnTyrLeuTyrAsnPheLeuGlyIleSerThrAspThrGlnIleArgTyrTyrThrGly
  421 AATTATTTATATAATTTTTTAGGTATAAGTACTGATACTCAAATAAGATATTATACAGGC

      IleAspAlaPheAsnGluIleArgLeuTyrValLysTyrGlyGlnLeuGlyTyrLysGly
  481 ATAGACGCTTTTAATGAAATAAGATTATATGTAAAATACGGACAATTAGGATATAAAGGC

      GlySerTyrThrAspLysSerTyrAspGluGluPhePheAlaArgSerPheGlyPheGlu
  541 GGTTCATATACGGATAAAAGTTATGATGAAGAATTTTTTGCAAGATCATTTGGTTTTGAA

      ThrArgPheTyrPheLeuAsnThrAlaValGlyAsnValThrIleAsnProPheIleLys
  601 ACAAGATTCTATTTTTTGAATACTGCTGTTGGAAATGTAACTATCAATCCTTTTATTAAA

      ValAlaTyrAsnThrAlaLeuHisGlyPheSerThrMetValArgSerLeuAspSerVal
  661 GTAGCATATAATACAGCTTTGCATGGATTTAGTACTATGGTAAGATCATTAGATAGTGTC

      IleGluGluIleGluGlyTyrSerSerAspArgThrAlaLysAlaAlaGlyAsnIleAsn
  721 ATTGAAGAAATAGAAGGTTATAGTTCAGATCGTACCGCTAAAGCAGCAGGAAATATTAAT

      AlaLysTrpAspLysAsnProTyrAspValThrValGlnAlaValLeuGlyValThrAla
  781 GCTAAATGGGATAAGAATCCTTATGATGTAACTGTGCAGGCAGTATTGGGAGTAACTGCT

      AsnSerAspIleValSerLeuTyrValGluProSerLeuGlyTyrArgAlaLysTyrLeu
  841 AATAGCGATATAGTATCACTTTATGTTGAGCCTTCTTTAGGTTATAGGGCTAAATATTTA

      GlyLysLeuThrTyrGluAspProAspGlyLysValAsnPheAspPheLysValAsnHis
  901 GGAAAATTAACATATGAAGATCCAGATGGAAAAGTTAATTTTGATTTTAAAGTTAATCAT

      TyrLeuSerTrpCysAlaTyrAlaGluLeuTyrIleThrProValLysAspLeuGluTrp
  961 TATTTATCTTGGTGTGCTTATGCAGAGCTTTATATAACACCTGTAAAAGATTTAGAATGG

      TyrPheGluMetAspValAsnAsnSerAspSerAspSerThrGlyIleProValSerPhe
 1021 TATTTTGAAATGGATGTTAATAATAGTGATTCAGATTCTACAGGTATACCTGTTAGTTTT
```

```
     AlaSerThrThrGlyIleThrTrpTyrLeuProGluPheOC     OC
1081 GCTTCTACTACAGGAATAACTTGGTATTTGCCAGAATTTTAATTATAAAGCAAATTTTAT

1141 ATGACAAAATAAAAAATGGGGCATTTATTATTAAAAAATAAATACCCCACATTTTATTAA

1201 ATAACTTCTTAAATAATTTTACA4TTTATATTTTATTAGTATAATAAAATATAAAGTTAA

1261 ATTTAGGTGTGTACAATGAAAAAAAGTTTTCTAATTATGACAGTATTATTAAGTATGTCA

                                                                End of
1321 TATTGTTCAATATTTGGTATGTATGGACATCAGGACGATTGGATTGATTTTCTTACAGTC

T. hyo. insert----->|
1381 GGTAATCAGTTTAGAGGGAATTC
```

What is Claimed is:

1. A protein capable of eliciting at least one antibody capable of recognizing at least one epitope of at least one T. hyo. antigen having a molecular weight of about 39 kDa.

2. The protein of Claim 1 encoded by gene 1.

3. The protein of Claim 2 encoded by the full length gene 1.

4. The protein of Claim 1 encoded by gene 2.

5. The protein of Claim 1 encoded by gene 3.

6. The protein of Claim 1 encoded by gene 4.

7. The protein of Claim 1 encoded by gene 5.

8. The protein of Claim 1 encoded by gene 6.

9. The protein of Claim 1 encoded by gene 7.

10. The protein of Claim 1 encoded by gene 8.

11. The protein of Claim 1 encoded by the full length gene 2.

12. The protein of Claim 1 encoded by the full length gene 3.

13. The protein of Claim 1 encoded by the full length gene 4.

14. The protein of Claim 1 encoded by the full length gene 5.

15. The protein of Claim 1 encoded by the full length gene 6.

16. The protein of Claim 1 encoded by the full length gene 7.

17. The protein of Claim 1 encoded by the full length gene 8.

18. DNA encoding at least one protein capable of eliciting at least one antibody recognizing at least one epitope of at least one T. hyo. antigen having a molecular weight of about 39 kDa.

19. The DNA of Claim 16 wherein said DNA is selected from the group consisting of genes 1, 2, 3,

4, 5, 6, 7 and 8 encoding a T. hyo. 39 kDa protein and mixtures thereof.

20. The DNA sequence of Claim 18 wherein the gene is a full length gene.

21. A host genetically engineered with DNA of Claim 18.

22. A host genetically engineered with DNA of Claim 19.

23. A host genetically engineered with DNA of Claim 20.

24. An expression vehicle including DNA of Claim 18.

25. An expression vehicle including the DNA of Claim 19.

26. An expression vehicle including the DNA of Claim 20.

27. Protein expressed by the host of Claim 21.

28. Protein expressed by the host of Claim 22.

29. Protein expressed by the host of Claim 23.

30. A process for protecting an animal against T. hyo., comprising:

administering to an animal to be protected an effective amount of at least one protein of Claim 1.

31. A process for protecting an animal against T. hyo., comprising:

administering to an animal to be protected an effective amount of at least one protein of Claim 27.

32. A vaccine for protecting an animal against T. hyo. comprising:

at least one protein of Claim 1 in conjunction with a pharmaceutically acceptable carrier.

33. A vaccine for protecting an animal against T. hyo. comprising:

at least one protein of Claim 27 in conjunction with a pharmaceutically acceptable carrier.

34. Antibody which recognizes T. hyo antigen having a molecular weight of about 39 kDa.

Figure #1--Cloning and sequencing of 39 kDa genes 1-8

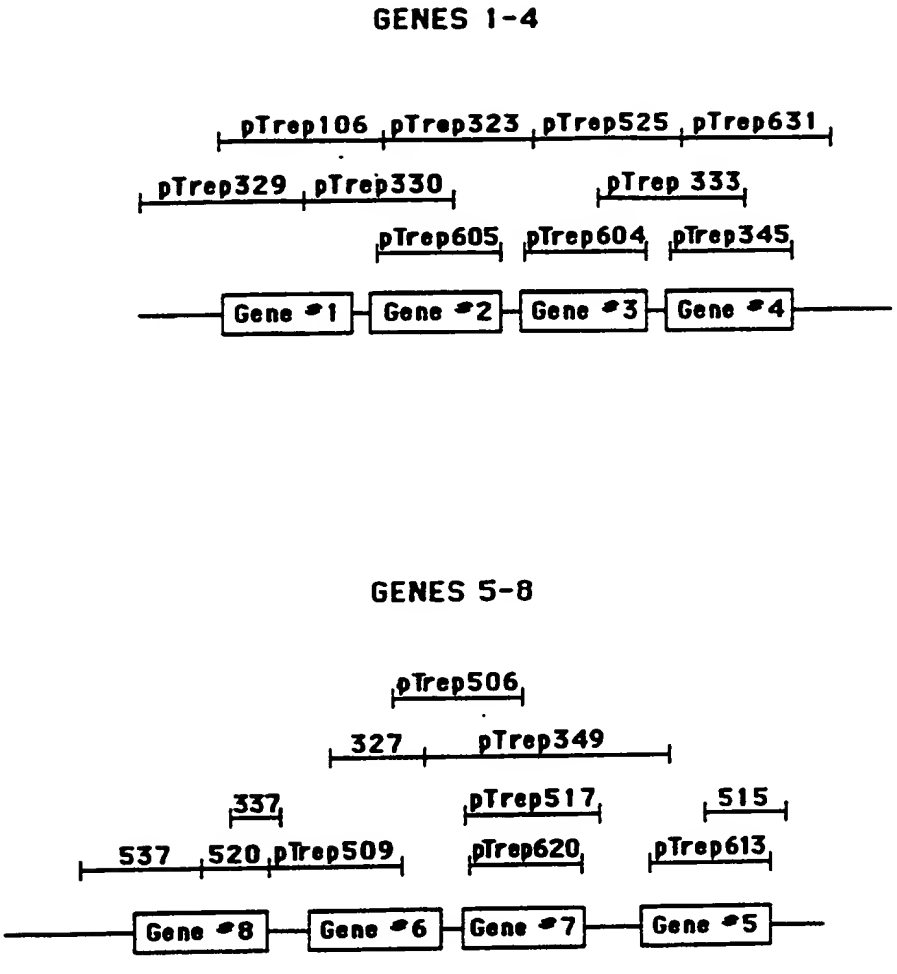


Figure #2--Schematic of pTrep505

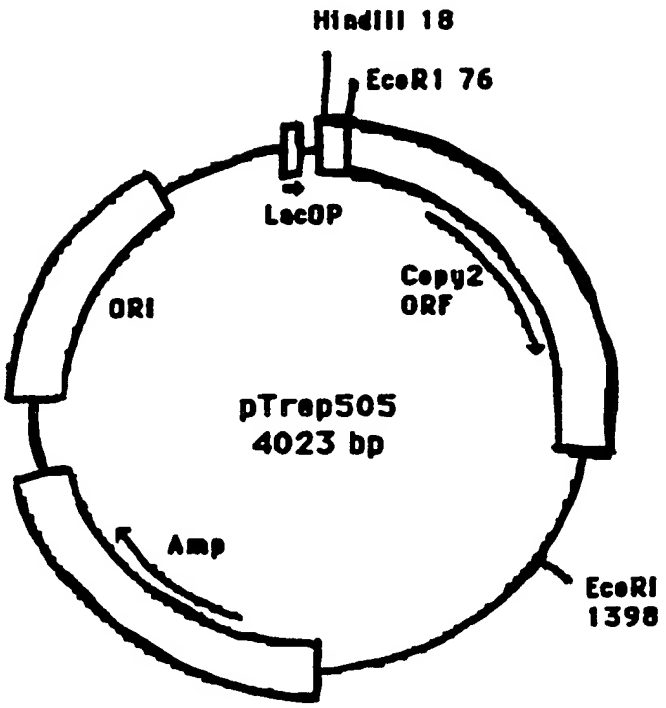


Figure #3--Construction of pTrep702

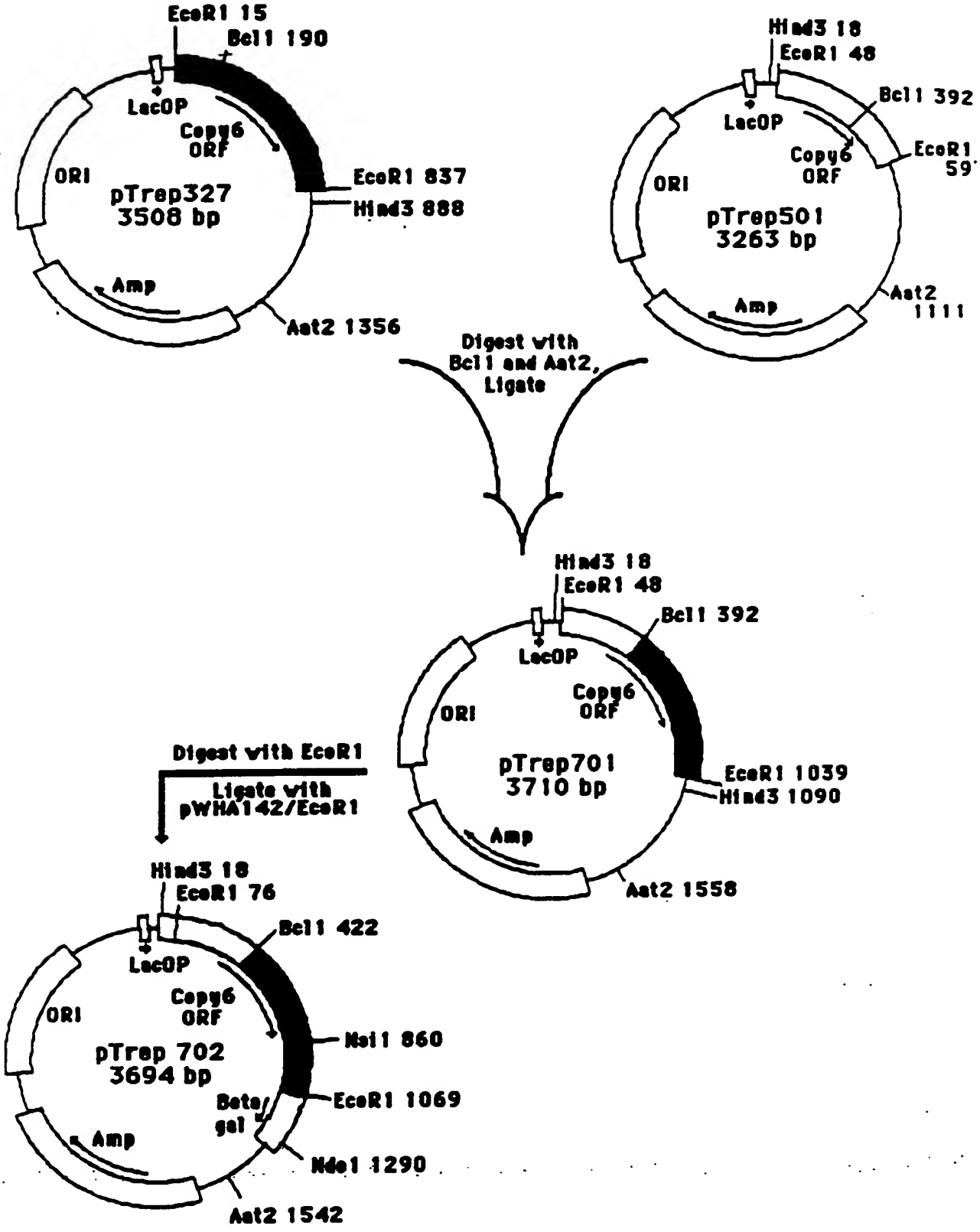


Figure #4--Construction of pTrep704

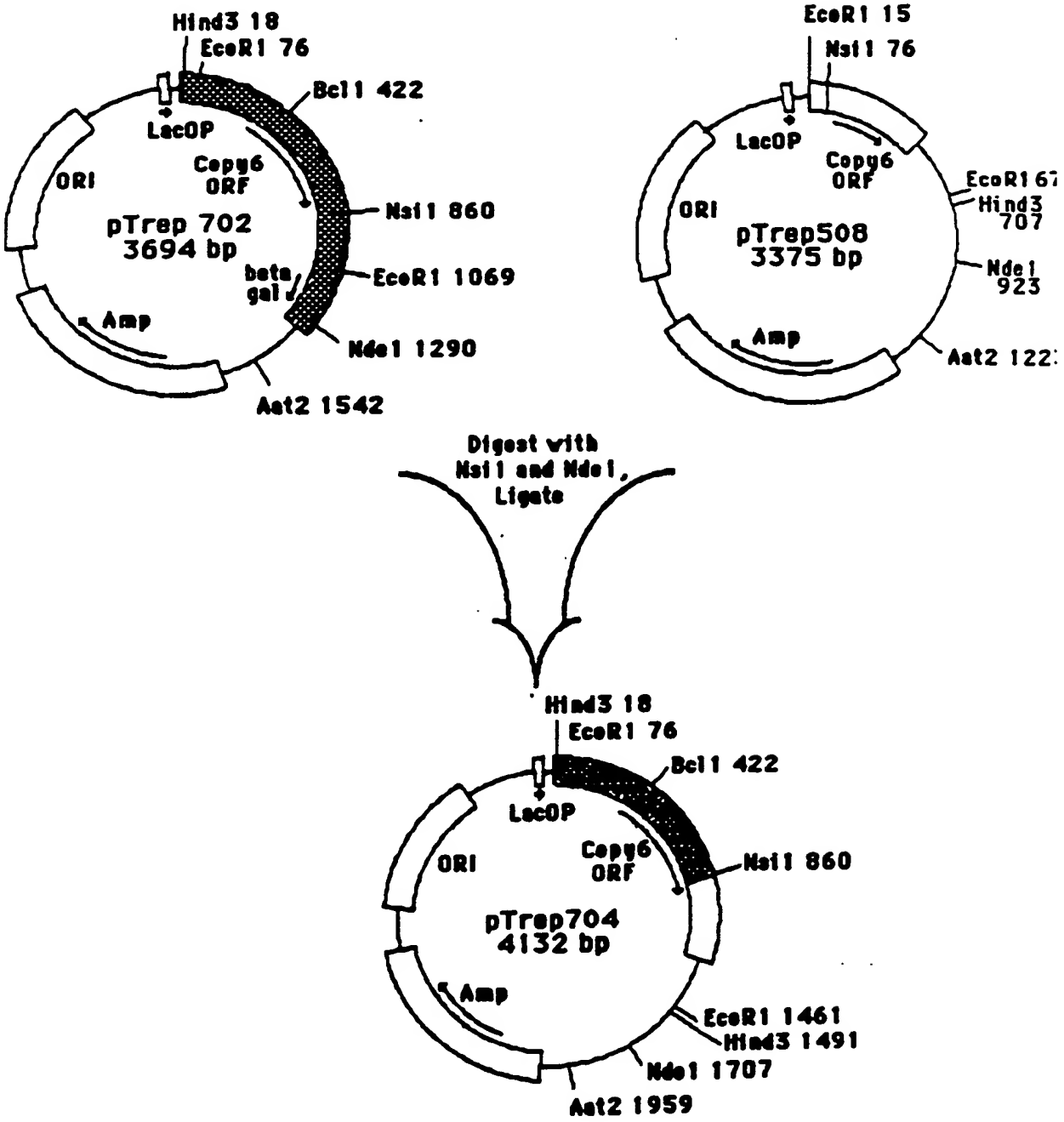
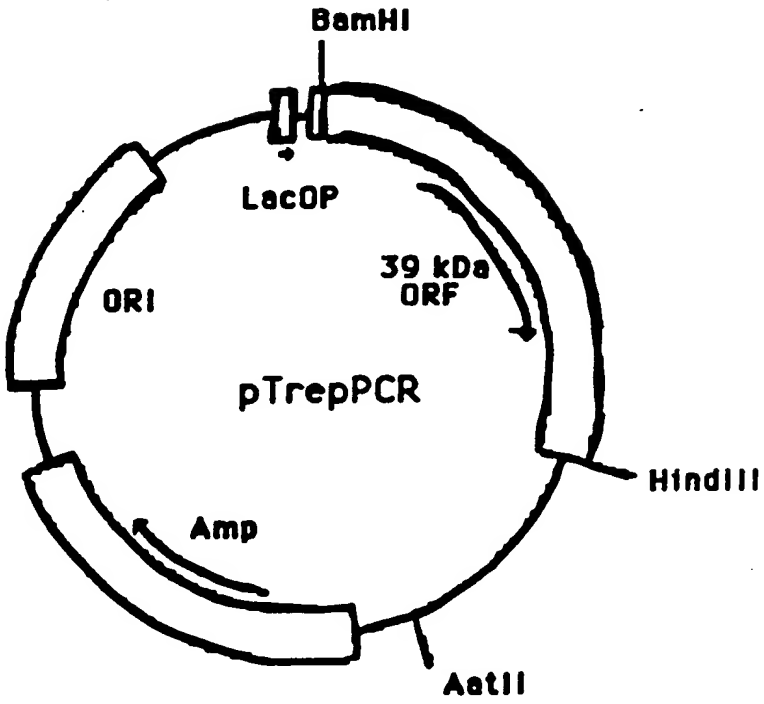


Figure #5--Schematic of PCR derived [illegible] expression clones



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